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Vertical distribution and migration of planktonic polychaete larvae in Onagawa Bay, north-eastern Japan

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Abstract

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The planktonic larvae of polychaetes are one of the most numerous and diverse groups in coastal zooplankton; however, little is known about their larval dynamics and the factors that affect their vertical distribution. We investigated the vertical distribution and migration of planktonic polychaete larvae in Onagawa Bay, north-eastern Japan, particularly focusing on the dominant spionid larvae. In total, 14 families of planktonic polychaete larvae and 14 species or genera of spionid larvae were identified during our study. Their density greatly fluctuated according to season and depth, with the polychaete larvae generally distributed in the lower layers of the water column. Furthermore, trends in vertical distribution of spionid larvae varied between species. In winter and spring, larvae of *Polydora onagawaensis* were the most prevalent, with a wide range in vertical distribution. In summer and autumn, larvae of *Pseudopolydora achaeta* and *Prionospio* spp. were the most prevalent spionid larvae and were primarily distributed in the lower layers of the water column. Trends in larval vertical distribution varied as a result of differences in adult habitat; these variations would enable the larvae to efficiently recruit into their appropriate adult habitats. Spionid larvae did not show diel vertical migration. Larvae of two spionid taxa, *Pseudopolydora achaeta* and *Prionospio* spp., exhibited tidal vertical migration, with larvae appearing to avoid dispersal by moving to slower-flowing deeper water during flood and ebb tides. Although many previous studies assume that, because of their limited swimming capacity, polychaete larvae are passively dispersed within the water column, this study indicates that polychaete larvae can control their vertical distribution to some extent, and this small-scale vertical migration may be important as a retention mechanism for polychaete larvae.

Keywords polychaete larva, Spionidae, *Polydora*, *Pseudopolydora*, *Prionospio*, vertical distribution, vertical migration, larval retention

Introduction

Many marine invertebrates pass through a planktonic larval phase during their early life history. Historically, larval dispersal has typically been considered a passive process, and most larvae have been thought to be unable to control their horizontal dispersal (Chia et al., 1984; Scheltema, 1986), with a few exceptions such as some larval crustaceans (Luckenbach and Orth, 1992). However, the ability of larvae to control their vertical distribution in the water column has been well known and can have significant outcomes in terms of larval transport and horizontal distribution, because the current speed and direction generally vary with depth (Young, 1995; Hill, 1998; Metaxas, 2001).

Tidal vertical migration patterns have been observed, particularly in estuarine invertebrate larvae (Carriker, 1951; Cronin, 1982). Tidal currents move faster at the surface layers and slower at the bottom layers because of the friction at the bottom layers. Therefore, larvae can be transported towards the sea or shore or remain within the estuary by migrating to the surface or bottom layers, respectively, in synchronisation with tidal cycles (Forward and Tankersley, 2001; Tankersley et al., 2002; Gibson, 2003). These larval behaviours related to relocation are also known as 'selective tidal stream transport' (Greer Walker et al., 1978).

Diel vertical migration is also well known for many planktonic animals, including invertebrate larvae. Three patterns of diel vertical migration (DVM) have been observed for planktonic invertebrate larvae: (i) nocturnal (normal) DVM, with an ascent to a minimum depth at night and a descent to a maximum depth during the day; (ii) reverse DVM, with the ascent to a minimum depth during the day and the descent to a maximum depth at night; (iii) twilight DVM, with an ascent to the surface at sunset, a descent to deeper water around midnight, a second ascent to the surface in the early morning hours, followed by a final descent to deeper water at sunrise (Forward, 1988; Pearre, 2003). Although the latter two patterns are rare for invertebrate larvae (Young and Chia, 1987; Queiroga and Blanton, 2005), some larvae, particularly decapods, are sensitive to the diel light cycle (Forward et al., 1984). These behaviours occur in a wide range of planktonic animals and are considered to be predator avoidance behaviour because larvae alter their DVM patterns in the presence of predators (Bollens and Frost, 1991; Neill, 1992; Cohen and Forward, 2009).

In addition to the light and tidal cycles, gravity, temperature, oxygen, salinity, hydraulic pressure and chemicals from phytoplankton and predators are believed to influence larval vertical distribution and migration (Huntley and Brooks, 1982; Pires and Woollacott, 1983; Forward, 1988; Lass and Spaak, 2003). Furthermore, larval behavioural responses are also changeable depending on species, larval condition and feeding history (Thorson, 1946, 1964; Metaxas and Young, 1998a; Arellano et al., 2012). Mechanisms determining vertical distribution and migration of planktonic larvae are complex. Although the diverse vertical distribution and migration of many planktonic animals is well known, there is limited information about these behaviours in polychaete larvae.

Polychaetes are one of the major components of coastal macrobenthos in terms of species richness, density and total biomass (Ward and Hutchings, 1996). They play major roles in the marine food web and in the functioning of benthic communities by their activity in decomposition of organic matter and bioturbation (Aller, 1982; Tomiyama et al., 2005). The planktonic larvae of polychaetes are one of the most numerous and diverse groups of coastal zooplankton (Omel'yanenko and Kulikova, 2002). Despite the great importance of this group in marine ecosystems, the planktonic larval phase of polychaetes is still poorly understood.

Materials and methods

To reveal seasonal vertical distribution of planktonic polychaete larvae, sampling was performed from January to December 2012 at St. 1 (38°26'14.42" N 141°27'38.79" E; 21–23 m depth) in Onagawa Bay (fig. 1). Zooplankton samples were collected once a month from the surface down to 20 m in depth at 5-m intervals using an Iwaki MD-70R shipboard magnet pump (Iwaki Co., Ltd, Tokyo, Japan). A priming water tank and suction hose were connected to a magnet pump and were being primed seawater before pumping. The nozzle of the suction hose was attached to a 1.5-kg weight with wire and dropped to each depth. Approximately 100 L of seawater was pumped up onto the boat and filtered through a hand net with a mesh size of 110 μm, and the plankton samples were fixed with 5% neutralised formaldehyde solution. Planktonic polychaete larvae were identified and counted under a stereomicroscope. Vertical profiles of temperature and salinity were determined using a CTD RINKO-Profiler (JFE Advantech Co., Ltd, Kobe, Japan).

Chlorophyll (Chl) *a* concentration was measured once per month. Water samples were collected from the surface down to 20-m depth at 5-m intervals with a 5-L Van Dorn water sampler. Subsamples of 128 mL were taken at each depth and pre-filtered through 200-μm mesh onto a GF/F filter (average pore size 0.7 μm). After filtration, each filter was immediately covered by a quantitative filter and aluminium foil to protect it from light. Chl *a* was extracted from each filter by immersion in 90% acetone for 24 h in the dark at –20°C , and fluorescence was determined with a Turner Designs fluorometer by the method demonstrated by Yentsch and Menzel (1963).

Diel and tidal vertical distribution of planktonic polychaete larvae were examined by sampling at 0-, 5-, 10-, 15- and 20-m depths at 3-h intervals over a 21-h period. Zooplankton samples were collected at 8:00, 11:00, 14:00, 17:00, 20:00 and 23:00 on 20 August 2012 (spring tide) and 2:00 and 5:00 on 21 August 2012 (half tide) and treated in the same manner as has been described above. Vertical profiles of temperature, salinity and Chl fluorescence values were determined using a CTD RINKO-Profiler.

In order to analyse the relationships between larval and Chl vertical distribution, a weighted mean depth (WMD) for each vertical profile was calculated using the following equation (Rollwagen-Bollens et al., 2006):

$$\mathrm{WMD} = \frac{\sum(A_i \cdot Z_i)}{\sum(A_i)},$$

where *i* is each depth sampled, *A* is the density of polychaete larvae (individuals m^{-3}) or chlorophyll *a* concentration (μg L^{-1}), and *Z* is the sampling depth (m). Pearson's product–moment correlation coefficient (*r*) and associated significant probability (*P*) were calculated to examine the relationship between larval and Chl vertical distribution.

The significance of differences in larval vertical distribution of the dominant *Pseudopolydora achaeta* and *Prionospio* spp. in daytime vs. night-time and flood and ebb vs. high and low tide during the 21-h investigation were tested using the statistical test for differences in vertical plankton distributions in the presence of patchiness when replicate samples were available (Beet et al., 2003). Samples taken at different times were pooled into two sets of observations, daytime and night-time, and flood/ebb and high/low tide, and considered as replicates. Plankton profiles collected at 5:00 on 21 August were treated as night-time profiles because it was just after daybreak. The null hypothesis, that the shapes of the depth profiles of mean abundance are the same under all conditions (i.e. daytime, night-time, flood/ebb tide, and high/low tide) was tested using the following test statistic (Paul and Banerjee, 1998):

$$\mathrm{B} = n \sum_{i=1}^{T} \sum_{j=1}^{D} \frac{(\bar{Y}_{ij} - \hat{\mu}_{ij})^2}{\hat{\mu}_{ij}(1+\hat{c}\hat{\mu}_{ij})},$$

where *T* and *D* are the number of conditions and depths, respectively, $\bar{Y}_{ij}$ is the average density of *n* replicates for condition

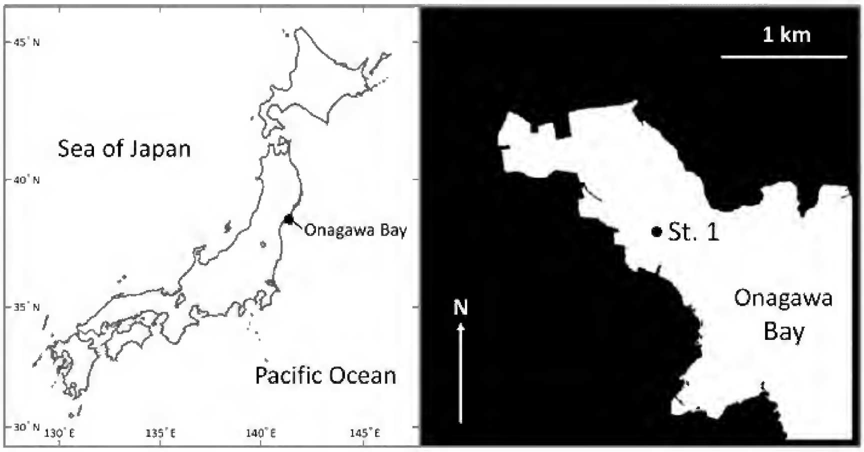

Figure 1. Location of the sampling station in Onagawa Bay.

i at depth j, and $\hat{\mu}_{ij}$ and $\hat{c}$ are the maximum likelihood (ML) estimates of the mean ($\hat{\mu}_{ij}$) and dispersion coefficient (c_{ij}) under the null hypothesis, respectively. The ML estimates, test statistic B, and its corresponding P-values under the null hypothesis were obtained using MATLAB software (MathWorks Japan, Tokyo, Japan), as described by Beet et al. (2003).

Results

Seasonal vertical distribution of planktonic polychaete larvae

Water temperature ranged from 4.4 to 25.9°C, with lowest temperatures at the 20-m depth in March and highest at 0-m depth in September. Thermal stratification in the water column began in April and lasted until September. The differences in temperature between the top and bottom waters were 2–5°C in these months. In other seasons, the water column was vertically well mixed. Salinity ranged from 28.4 to 34.7 but was generally stable at approximately 33–34. An episodic decrease in surface salinity in April was a consequence of heavy rainfall. Except for the 0-m depth, salinity was almost the same in all layers of the water column.

Chl *a* concentration varied from 0.18 to 11.71 μg L^{-1}, with marked seasonal and vertical variations (fig. 2). The lowest value was recorded at 20-m depth in June and the highest at 10-m depth in April. Typical of the annual pattern in temperate waters, there was a large spring phytoplankton bloom throughout the water column in February and another in April (fig. 2). High Chl *a* concentrations were also observed in the bottom water in January, July and August, and at 0 m in September. The Chl *a* concentration was very low in March, June, November and December.

The density of planktonic polychaete larvae fluctuated from 0 to 6240 individuals (ind.) m^{-3} and varied greatly according to season and depth (fig. 2). The lowest density was recorded at 0-m depth in February and March, and the highest density was recorded at 5-m depth in May. Larvae belonging to 14 families were identified. Spionidae was the most dominant family for most months (68.2%), followed by Phyllodocidae (11.6%) and Polynoidae (5.7%). In general, polychaete larvae were very sparse at the surface (0-m depth) and were distributed in the lower layers of the water column (fig. 2). This trend in vertical distribution was primarily observed in dominant spionid larvae, as well as in the larvae of Phyllodocidae and Terebellidae. In contrast, Serpulidae larvae tended to be located in the upper layers of the water column. There was slight correlation between weighted mean depth of planktonic polychaete larvae and Chl concentration during the study period from January to December 2012, but this correlation was not significant ($r = 0.44$, $P = 0.149$; $n = 12$).

The density of planktonic spionid larvae fluctuated from 0 to 5680 ind. m^{-3}, and also differed greatly depending on season and depth (fig. 3). The lowest densities were recorded at 0-m depth in February, March and December, and the highest density was recorded at 5-m depth in May. Larvae belonging to 14 species/genera were identified. *Pseudopolydora achaeta* was the dominant species (36.7%), followed by *Polydora onagawaensis* (30.6%) and *Prionospio* spp. (10.7%). During the period from January to June, larvae of the spionid *P. onagawaensis* were dominant. They did not show specific trends with regard to vertical distribution and tended to be distributed at a wide range of depths. During summer, larvae of *Pseudopolydora achaeta* and *Prionospio* spp. were the dominant species/genus and tended to be distributed in the lower layers of the water column.

Diel and tidal vertical distribution of planktonic polychaete larvae

Water temperature ranged from 18.0 to 23.5°C, with the lowest temperature near the bottom and the highest at the surface. There was thermal stratification, and a stable thermocline was observed between 0- and 5-m depths for the entire period. Salinity ranged from 32.3 to 33.9 and was stable around 33.0 to 34.0, except in the surface water at 11:00 on 21 August. Except for the 0-m depth, salinity was almost the same in all layers of the water column. Chlorophyll fluorescence values

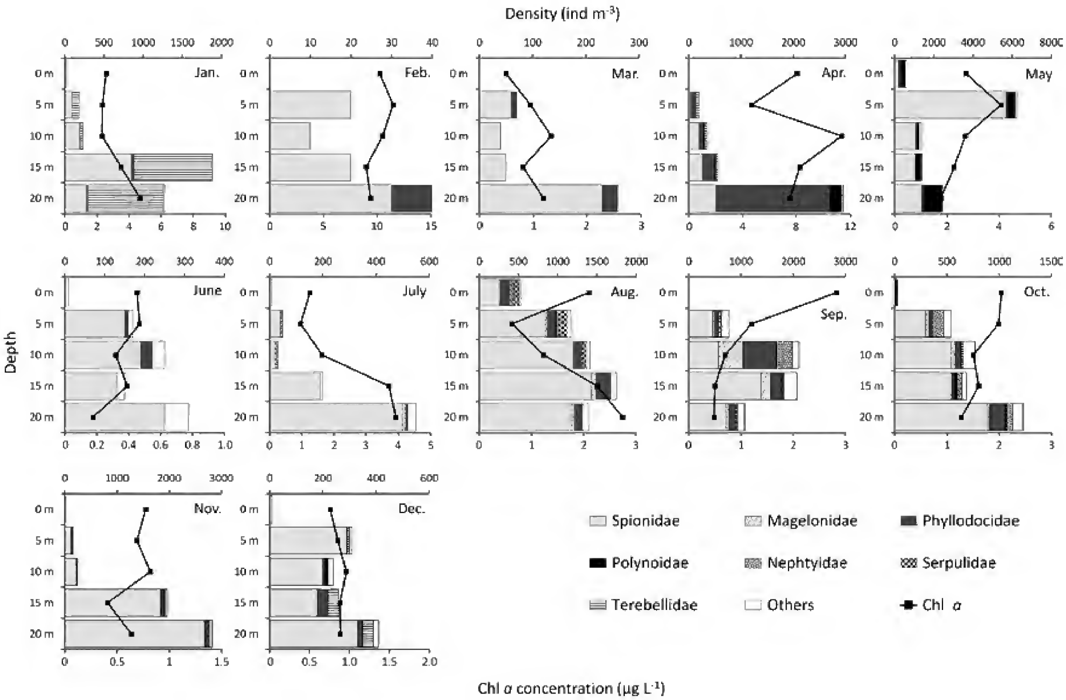

Figure 2. Vertical distribution of each family of planktonic polychaete larvae (upper axes) and chlorophyll *a* concentration (μg L^{-1}) (lower axes) at St. 1 in Onagawa Bay from January to December 2012.

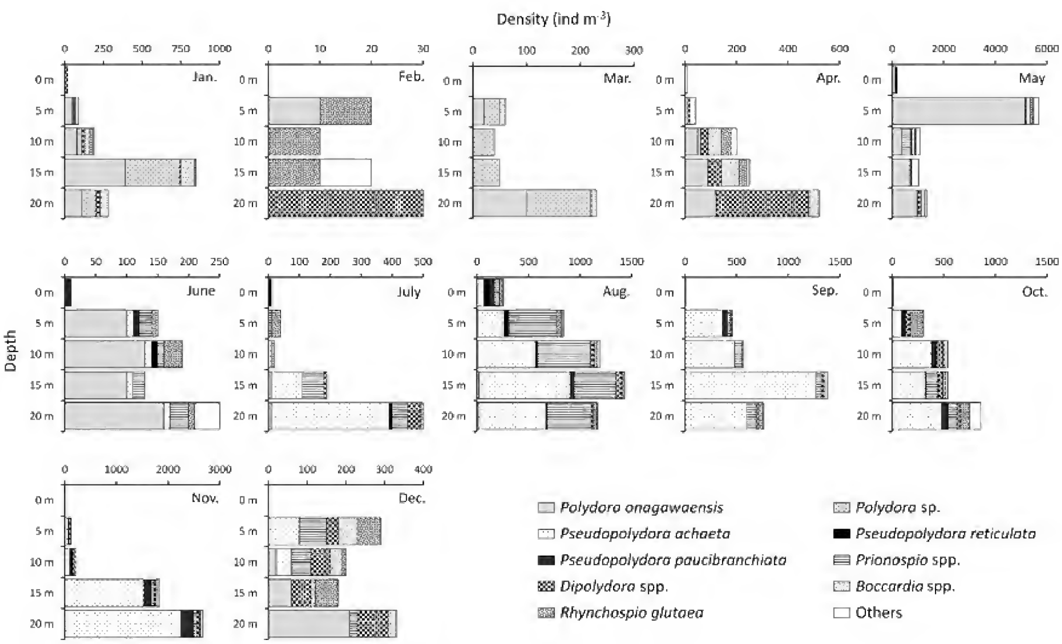

Figure 3. Vertical distribution of each species or genus of planktonic spionid larvae at St. 1 in Onagawa Bay from January to December 2012.

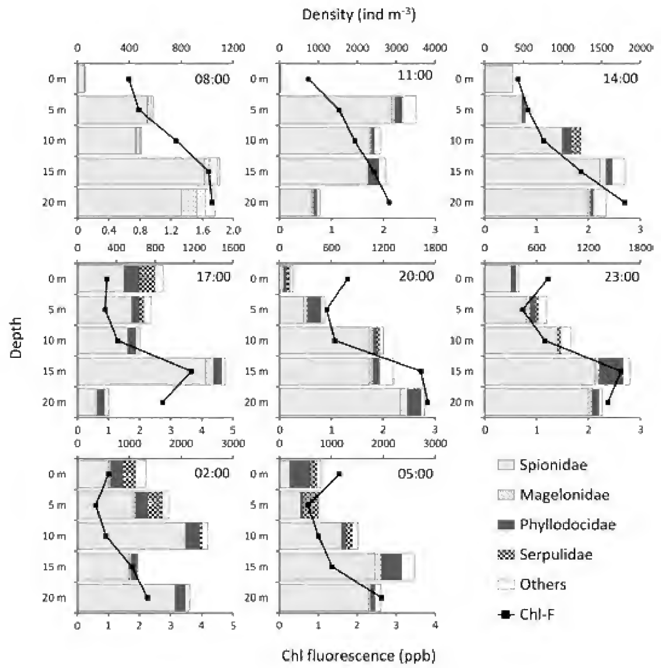

Figure 4. Diel changes in vertical distribution of planktonic polychaete (upper axes) and chlorophyll fluorescence (ppb) (lower axes) larvae at St. 1 in Onagawa Bay from 8:00 a.m. on 20 August to 5:00 a.m. on 21 August, 2012.

ranged from 0.6 to 3.7 ppb, with the lowest value at 0-m depth at 11:00 and the highest at 15-m depth at 17:00 (fig. 4). The maximum chlorophyll levels were found deeper in the water column all throughout the day.

The density of planktonic polychaete larvae fluctuated from 40 to 3520 ind. m^{-3} and varied greatly according to depth (fig. 4). The lowest density was recorded at the 0-m depth at 11:00, and the highest density was recorded at the 5-m depth at 11:00. Larvae belonging to 11 families were identified on 20 and 21 August. Spionidae was the dominant family at all times (78.9%), followed by Phyllodocidae (9.3%) and Serpulidae (3.6%). The larvae of Spionidae and Magelonidae were almost absent at the surface (0-m depth) and tended to be distributed in the lower layers of the water column. In contrast, the larvae of Serpulidae tended to be distributed in the upper layers of the water column.

Planktonic spionid larval densities ranged from 30 to 2880 ind. m^{-3} (fig. 5). The lowest density was at 0-m depth at 11:00 and the highest density at 5-m depth at 11:00. Larvae belonging to 11 species/genera were identified. *Pseudopolydora achaeta* and *Prionospio* spp. were the dominant species/genera (49.9% and 38.3%, respectively). In general, spionid larvae were sparse at the surface (0 m) and tended to be distributed in the lower layers of the water column. However, the highest density was recorded at the 5-m depth at 11:00 because of the extremely high density of *Prionospio* spp. (2040 ind. m^{-3}). The larvae of *Pseudopolydora achaeta* and *Prionospio* spp. tended to distribute slightly shallower during high and low tide and deeper during flood and ebb tide, especially in daylight hours (fig. 6). However, there were no statistically significant differences in the vertical distribution of *Pseudopolydora achaeta* and *Prionospio* spp. during the day vs. night ($B = 1.39$, $P > 0.05$ and $B = 8.23$, $P > 0.05$, respectively), or flood/ebb vs. high/low tide ($B = 1.38$, $P > 0.05$ and $B = 3.20$, $P > 0.05$).

Discussion

Vertical distribution of planktonic polychaete larvae

Polychaete larvae in Onagawa Bay generally tend to be distributed at higher densities in the lower layers of the water column and sparser densities at the surface (figs 2 and 4). In Onagawa Bay, the close timing between the phytoplankton blooms and the occurrence of planktonic polychaete larvae had been observed previously, and most planktonic polychaete larvae have tended to synchronise with summer phytoplankton increases and fall blooms (Abe et al., 2011). Because the phytoplankton increase near the surface during summer and autumn in Onagawa Bay, it was previously assumed that a photopositive response brought larvae up towards the phytoplankton-rich surface waters, as indicated by Thorson (1946; 1964) during summer and autumn in Onagawa Bay (Abe et al., 2011). However, the results of this study contradicted this assumption, because they indicated that polychaete larvae tended to be distributed in the lower layers rather than the

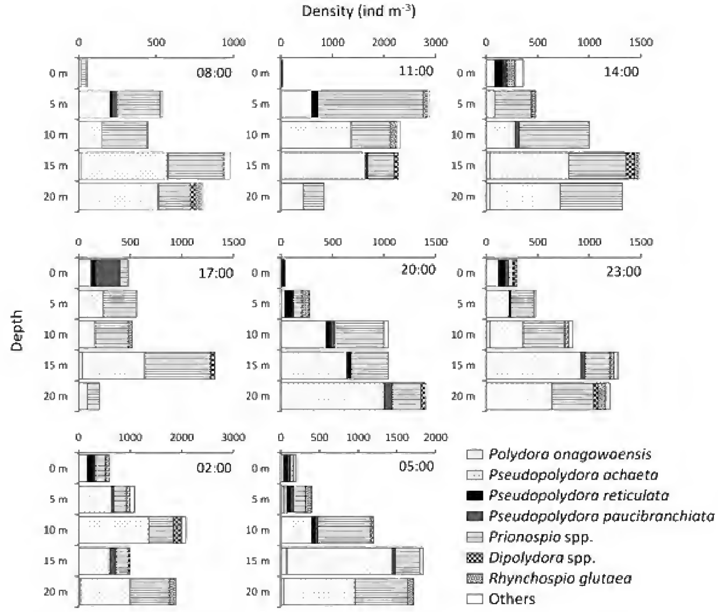

Figure 5. Diel changes in vertical distribution of planktonic spionid larvae at St. 1 in Onagawa Bay from 8:00 a.m. on 20 August to 5:00 a.m. on 21 August 2012.

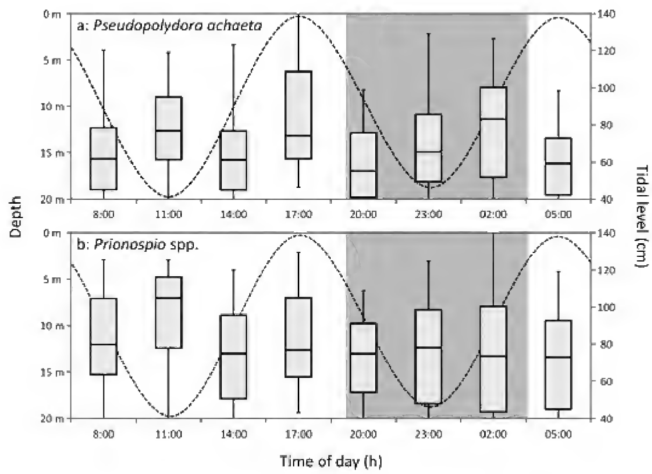

Figure 6. Box plots of vertical distribution of two spionid larvae: a, *Pseudopolydora achaeta* and b, *Prionospio* spp. The central line in the box represents the median, the upper and lower boundaries of the box represent the quartiles, and the vertical bar represents the 95% range of larval distribution (left axes). The dashed wavy lines and dark shaded areas represent the tidal level (right axes) and night-time, respectively.

surface layers, even in summer and autumn. The previously documented positive phototactic response of spionid larvae (Blake and Woodwick, 1975; Levin, 1986) was easily observed in this study, as the live larvae clearly moved towards the light source during microscope observation. However, this photopositive behaviour did not result in vertical migration in the field, as we found spionid larvae distributed primarily in the lower layers. The majority of young larvae of the benthic invertebrates were reported to be positively phototactic under laboratory illumination (Thorson, 1946) but have been

observed to avoid very strong light and often to be absent from the surface layers of the sea (Russell, 1927; Thorson, 1964). The results of our study appear to be consistent with these reports. However, the results of our 21-h survey showed that the vertical distribution of spionid larvae did not vary between daytime and night-time (figs 4–6); therefore, strong light intensity cannot be the explanation for the scarcity of spionid larvae at the surface. In other studies, polychaete larvae have been found in higher densities at the bottom of the water column (Wilson, 1982; Ambrogi et al., 1989; Yokoyama, 1995; Schlüter and Rachor, 2001). Distribution of polychaete larvae in the bottom layers of the water column may be a common phenomenon in many marine waters.

In contrast to most of the polychaete larvae that were present in the lower layers of the water column, only Serpulidae larvae, one of the predominant intertidal animals in Onagawa Bay, tended to be distributed in the upper layers of the water column (figs 2 and 4). Thorson (1964) generalised that the larvae of intertidal species are photopositive throughout their planktonic larval period and larvae of most subtidal species are initially photopositive but become photonegative before settlement. Although it is unknown if the difference in larval vertical distribution between intertidal and subtidal polychaetes is due to a difference in their phototactic response, the difference in larval vertical distribution may be reflected in their adult habitats.

The vertical distribution trend of spionid larvae differed from species to species in this study. The larvae of *Pseudopolydora achaeta* and *Prionospio* spp. tended to be distributed in the lower layers of the water column (figs 3 and 5), whereas *P. onagawaensis* larvae showed no specific trends in vertical distribution and were distributed at a wide range of depths (fig. 3). *Polydora onagawaensis*, a recently described species from Onagawa Bay (Teramoto et al., 2013), is a shell-boring polychaete, and adults inhabit the shells of molluscs distributed in the intertidal zone as well as those suspended in deeper water for aquaculture in Onagawa Bay. It is possible that the larval distribution of *P. onagawaensis* is determined by the habitat in which the larvae were produced and hatched, and their wide range of vertical distribution has a role in larval recruitment to the vertically wide range of adult populations. The larvae of *Pseudopolydora reticulata* and *Rhynchospio glutaea* tended to be distributed in the surface and middle layers of the water column, respectively (figs 3 and 5). Adults of *Pseudopolydora reticulata* and *R. glutaea* are commonly distributed in the soft bottom sediments of intertidal and shallow subtidal zones (Radashevsky and Hsieh, 2000; Zhou et al., 2010). Shallower distribution of these larvae was also consistent with the area in which they were produced and probably assists with larval recruitment to adult populations. Larval vertical distribution may be influenced by the water layer in which hatching occurred (Pearse, 1994).

Some invertebrate larvae have been observed to alter their swimming behaviour in response to the presence and quality of food patches (Raby et al., 1994; Metaxas and Young, 1998b; Burdett-Coutts and Metaxas, 2004). In this study, although there was no significant correlation, a similar trend in vertical distribution between polychaete larvae and Chl *a* concentration was observed in several months (fig. 2). It is considered that various factors influence larval vertical distribution, so it would be difficult to detect a clear correlation between larval and Chl vertical distribution. However, the observed trend may indicate that the vertical distribution of Chl regulates the vertical distribution of polychaete larvae to some extent in Onagawa Bay.

Diel and tidal vertical migration of planktonic polychaete larvae

The phenomenon of DVM widely occurs in many marine zooplankton taxa (Rawlinson et al., 2004), including polychaete larvae (Garland et al., 2002). However, no difference was observed in the vertical distribution of polychaete larvae between the light and dark hours over the 21-h sampling period in Onagawa Bay (figs 4–6). Polychaete larvae did not show DVM in this study indicates that the light condition was not very important for larval vertical distribution of polychaete larvae. This was consistent with the results of seasonal vertical distribution of spionid larvae in this study.

It is well known that some invertebrate larvae show a tidal vertical migration pattern (Cronin, 1982). In this study, although there was no significant relationship between larval vertical distribution and tidal cycle, the larvae of *Pseudopolydora achaeta* and *Prionospio* spp. tended to be distributed at slightly shallower depths during high and low tide and at greater depths during flood and ebb tide, especially in daylight hours (fig. 6). In general, tidal currents are faster at the surface layers and slower at the bottom layers. Therefore, these larval tidal migrations were considered to avoid dispersal by moving to slower-flowing deeper water during flood and ebb tide. Tidal vertical migration has also been reported in the larvae of the colony-forming polychaete *Sabellaria alveolata*; the larvae of *S. alveolata* tended to migrate closer to the surface during flood tide and nearer to the bottom during ebb tide, promoting a net landward transport of larvae (Dubois et al., 2007). Although the swimming capacity of polychaete larvae is often limited, and vertical migration was small, this vertical migration may be important as a retention mechanism for polychaete larvae.

Some larvae are reported to vary their DVM behaviour throughout ontogeny (Neill, 1992). Although ontogenic migration is not a general feature in polychaete larvae, ontogenic larval migration has been reported in two polychaete species, *Pectinaria koreni* and *Owenia fusiformis* (Lagadeuc et al., 1990; Thiebaut et al., 1992), and these ontogenic migrations are believed to be important for larval retention in the estuarine and coastal environments. Larval vertical migration is well known for decapod larvae, bivalve larvae and gastropod larvae (Cronin, 1982; Forward et al., 1984; Forward and Tankersley, 2001; Gibson, 2003; Rawlinson et al., 2004; Lloyd et al., 2012). Meanwhile, many studies assume a passive dispersal of polychaete larvae within the water column (Banse, 1986; Stancyk and Feller, 1986; Levin, 1986; Kingsford et al., 2002). There is very little information on the vertical distribution and vertical migration of polychaete larvae throughout the world, and this study indicates the need for additional knowledge about the vertical migration of polychaete larvae.

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Ampharete undecima, a new deep-sea ampharetid (Annelida, Polychaeta) from the Norwegian Sea

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Abstract Alvestad, T., Kongsrud, J.A. and Kongshavn, K. 2014. *Ampharete undecima*, a new deep-sea ampharetid (Annelida, Polychaeta) from the Norwegian Sea. *Memoirs of Museum Victoria* 71: 11–19.

Ampharete undecima, a new deep-sea polychaete belonging to the family Ampharetidae, is described from slope depths in the Norwegian Sea. The new species is of small size, up to 5 mm long and 0.5 mm wide, and thus it may have been overlooked in previous studies. It is shown to be a common and widespread species in the Nordic Seas in depths ranging from 600–1650 m. The new species is referred to the genus *Ampharete* based on characteristics of the prostomium, the presence of buccal tentacles with secondary pinnulae, four pairs of branchiae arising from fused segment II + III, 12 thoracic uncinigerous segments, and a single pair of nephridial papillae on segment IV. The new species differs from all known species of *Ampharete* in having 11 rather than 12–28 abdominal uncinigerous segments.

Keywords MAREANO, Nordic Seas, Arctic, Norway, Ampharetidae, PolyNor, new species

Introduction

The genus *Ampharete* Malmgren, 1866, as defined by Jirkov (2011), is a species-rich genus of sediment-dwelling polychaetes, comprising about 40 nominal species worldwide (Parapar et al., 2012). The Northern Atlantic and Arctic species of *Ampharete* have been well studied by several authors, including Holthe (1986), Jirkov (1997, 2001) and Parapar et al. (2012). However, information about the occurrence and distribution of *Ampharete* in the deeper parts of the Nordic Seas is still inadequate, and taxonomic challenges were indicated by Jirkov (2001). The water masses below ~650 m depth in the Nordic Seas are of Arctic origin, with temperatures below 0°C, and differ significantly from the relatively warm surface waters, which are of Atlantic origin (Blindheim and Østerhus, 2005). A major shift in species diversity and composition in the Nordic seas related to the different water masses has been indicated for several invertebrate taxa, including polychaetes (Svavarsson et al., 1993; Høisæter, 2010; Kongsrud et al., 2011; Bakken et al., 2014).

The present study is based on material from a large number of samples from deep-water habitats in the Nordic Seas collected during several cruises with RV *H. Mosby* in the 1980s (organised by the University of Bergen) and from the ongoing large-scale mapping program MAREANO (Marine AREAl database for NOrwegian waters, 2013). During general identification work of polychaetes from widespread deep-sea samples from the Nordic Seas, numerous specimens representing an undescribed species of *Ampharete* were encountered. The new species is of diminutive size (less than 5 mm in length) and may thus have been overlooked in previous studies. In the present study, we formally describe this new species of Ampharete utilising scanning electron microscopy to study and illustrate morphological characteristics. Further, based on presence or absence of the new species in a large number of deep-sea samples from the Nordic Seas, we describe the occurrence and distribution of the new species in the area.

Materials and methods

A large portion of the material used in the present study originates from several cruises with RV *H. Mosby* in the period 1981–1987 to different areas of the Nordic Seas (see Kongsrud et al. (2011) for details), collected using an RP-sledge (Brattegard and Fosså, 1991). The MAREANO samples were collected in 2008 and 2009 from off the north-west coast of Norway using an RP-sledge and a van Veen grab (0.2 m^2) (MAREANO 2013). The remaining few samples were

collected in 1990 during the RV *Meteor* cruise west of Bear Island at about 75°N using an RP-sledge, and from environmental monitoring off the west coast of Norway collected using a box corer. All sampling localities are shown in fig. 1. Geographical positions are given in decimal degrees.

All samples included in the present study have been washed through sieves with a mesh size of 0.5 mm. The materials have been prefixed in 10% formaldehyde and subsequently transferred to 75% alcohol. All examined specimens are deposited in the Natural History Collections, University Museum of Bergen, Norway (ZMBN).

The specimens were identified using dissecting and compound microscopes. Staining with methyl blue has been used to aid in identification. Line drawings of the holotype were prepared using a dissecting microscope with a camera lucida. SEM images were made using a ZEISS Supra 55VP microscope at the Laboratory for Electron Microscopy, University of Bergen.

Systematics

Family **Ampharetidae** Malmgren, 1866

Genus ***Ampharete*** Malmgren, 1866

Ampharete undecima sp. nov.

Zoobank LSID. http://zoobank.org/urn:lsid:zoobank.org:act:405975B9-C3FF-4CEE-92FF-44C0A3671AAD

Figures 2–6

Type locality. Norwegian Sea, 72.367°N 14.895°E, 770 m depth.

Type material. RV *G.O. Sars* MAREANO stn R379-47, RP, 9 Apr 2009, holotype (ZMBN 94022), 2 paratypes mounted for SEM (ZMBN 94023), 19 paratypes (ZMBN 94024), 19 paratypes (ZMBN 94025) and 1 paratype (ZMBN 94026).

Additional material. RV *H. Mosby*: Stn 81.03.21.1, 63.166°N 4.816°E, 830 m, 21 Mar 1981 (1 spec.); stn 81.06.04.4, 66.983°N 4.270°E, 1380 m, 4 Jun 1981 (1); stn 81.06.06.7, 65.716°N 5.238°E, 794 m, 6 Jun 1981 (34); stn 81.06.06.8, 65.666°N 4.815°E, 996 m, 6 Jun 1981 (9); stn 81.08.16.3, 62.800°N 1.043°E, 1009 m, 16 Aug 1981 (3); stn 82.01.21.2, 62.491°N 1.721°E, 604 m, 21 Jan 1982 (6); stn 82.01.21.4, 62.560°N 0.981°E, 804 m, 21 Jan 1982 (5); stn 82.01.21.6, 62.803°N 1.088°E, 984 m, 21 Jan 1982 (5); stn 82.08.15.1, 63.048°N 0.808°E, 1286 m, 15 Aug 1982 (2); stn 82.08.23.1, 63.213°N 3.121°E, 1003 m, 23 Aug 1982 (3); stn 82.11.26.1, 63.178°N 2.765°E, 1030 m, 26 Nov 1982 (1); stn 82.11.27.1, 62.985°N 3.218°E, 804 m, 27 Nov 1982 (73); stn 83.06.02.1, 62.198°N 0.003°W, 708 m, 2 Jun 1983 (15); stn 83.06.03.2, 60.201°N 6.625°W, 1220 m, 3 Jun 1983 (55); stn 83.06.08.1, 65.168°N 9.493°W, 784 m, 8 Jun 1983 (4); stn 83.06.08.2, 65.460°N 7.588°W, 1626 m, 8 Jun 1983 (3); stn 83.06.17.2, 62.338°N 1.411°W, 543 m, 17 Jun 1983 (1); stn 83.06.17.3, 62.593°N 1.233°W, 781 m, 17 Jun 1983 (18); stn 84.05.23.1, 62.585°N 1.793°W, 656 m, 23 May1984 (328); stn 84.05.23.3, 62.508°N 1.851°W, 576 m, 23 May 1984 (5); stn 84.05.23.7, 62.411°N 1.540°W, 575 m, 23 May 1984 (2); stn 84.11.20.2, 63.133°N 1.895°W, 1087 m, 20 Nov 1984 (29); stn 84.11.21.1, 62.791°N 1.836°W, 811 m, 21 Nov 1984 (2); stn 85.01.08.1, 62.525°N 1.443°W, 701 m, 08 Jan 1985 (135); stn 85.01.08.2, 62.706°N 1.186°W, 897 m, 08 Jan 1985 (44); stn 85.01.12.2, 63.166°N 0.643°W, 1489 m, 12 Jan 1985 (1); stn 85.01.12.3, 63.048°N 0.796°W, 1293 m, 12 Jan 1985 (1); stn 86.06.13.1, 63.218°N 7.031°W, 1261 m, 13 Jun 1986 (1); stn 86.07.25.1, 69.023°N 8.410°W, 879 m, 25 Jul 1986 (10); stn 86.07.27.2, 70.810°N 9.728°W, 886 m, 27 Jul 1986 (6); stn 86.08.15.5, 62.610°N 1.573°W, 654 m, 15 Aug 1986 (26); stn 86.08.15.7, 62.843°N 1.431°W, 951 m, 15 Aug 1986 (15); stn 86.08.17.5, 62.996°N 1.140°W, 1143 m, 17 Aug 1986 (4); stn 86.08.17.6, 62.691°N 1.756°W, 750 m, 17.08.1986 (115). RV *Meteor*: Stn M410/90, 74.843°N 15.377°W, 894 m, 16 Jul 1990 (79); stn M507/90, 74.883°N 15.275°W, 991 m, 28 Jul 1990 (87). RV *G.O. Sars* MAREANO: Stn R209-17, GR, 69.800°N 16.420°W, 1590 m, 5 Jun 2008 (1); stn R209-18, GR, 69.800°N 16.420°W, 1590 m, 5 Jun 2008 (1); stn R229-27, GR, 69.142°N 13.682°W, 1115 m, 11 Jun 2008 (1); stn R232-34, GR, 69.407°N 14.696°W, 1408 m, 14 Jun 2008 (1); stn R297-346, GR, 68.653°N 11.908°W, 807 m, 14 Oct 2008 (3); stn R297-347, GR, 68.653°N 11.908°W, 807 m, 14 Oct 2008 (1); stn R351-355, GR, 68.840°N 13.087°W, 765 m, 29 Oct 2008 (2); stn R351-356, GR, 68.840°N 13.087°W, 765 m, 29 Oct 2008 (2); stn R379-363, GR, 72.367°N 14.895°W, 760 m, 9 Apr 2009 (5); stn R379-47, RP, 72.367°N 14.895°W, 770 m, 9 Apr 2009 (10); stn R391-370, GR, 72.278°N 15.666°W, 729 m, 12 Apr 2009 (5); stn R391-51, RP, 72.281°N 15.666°W, 728 m, 12 Apr 2009 (34); stn R397-54, RP, 72.247°N 15.945°W, 635 m, 14 Apr 2009 (3); stn R404-381, GR, 72.078°N 15.806°W, 621 m, 15 Apr 2009 (1); stn R405-59, RP, 72.140°N 15.346°W, 899 m, 20 Apr 2009 (20); stn R406-61, RP, 72.189°N 14.829°W, 1030 m, 21 Apr 2009 (20); stn R444-148, RP, 71.741°N 15.236°W, 993 m, 20 Sep 2009 (7); stn R776-51, BC, 68.189°N 10.362°W, 873 m, 3 May 2012 (1). Environmental monitoring: Stn V-12, 67.002°N 5.334°W, 1330 m, 1 Jun 1998 (2).

Diagnosis. A small species of up to 5 mm in length and 0.5 mm in width. Branchiae arranged close together; three pairs in anterior transverse row and last pair in a posterior position. Paleae long, thin and slender with curved tips, 9–12 on each side. Abdomen with 11 chaetigerous segments. Pygidium with two short conical lateral cirri and a number of small rounded papillae.

Description. Holotype, complete, 4 mm long and 0.4 mm wide in thorax (fig. 2A–B). Other complete specimens are up to 5 mm in length and 0.5 mm in width. Colour in alcohol pale yellow.

Prostomium trilobed, without glandular ridges or eyes; prostomial median lobe delimited by deep lateral grooves, widest at the base, gradually narrowing to form acute, rounded frontal part (fig. 3A–B). Paired nuchal organs as circular, ciliated spots located in lateral grooves at base of median prostomial lobe (fig. 3B). Buccal tentacles with secondary filaments, pinnae; tips of pinnae covered by tufts of cilia (fig. 4B–C). Four pairs of long branchiae on fused segment II+III; three pairs of branchiae arranged in anterior, transverse row without median gap, fourth pair slightly posterior to anterior row, between 2nd outermost and innermost branchiae of anterior row (fig. 2C). Bases of branchiae in anterior row completely fused, forming a characteristic and well-marked edge above head in frontal view (fig. 3A–B). Branchiae of segment II in 2nd outermost position of anterior row, branchiae of segment III in outermost position of anterior row, branchiae of segment IV in innermost position of anterior row, branchiae of segment V in posterior position (figs 2C, 4A). One pair of nephridial papillae, located dorsally between the two posterior branchiae on segment IV (figs 2C, 4A). Fused segment II and

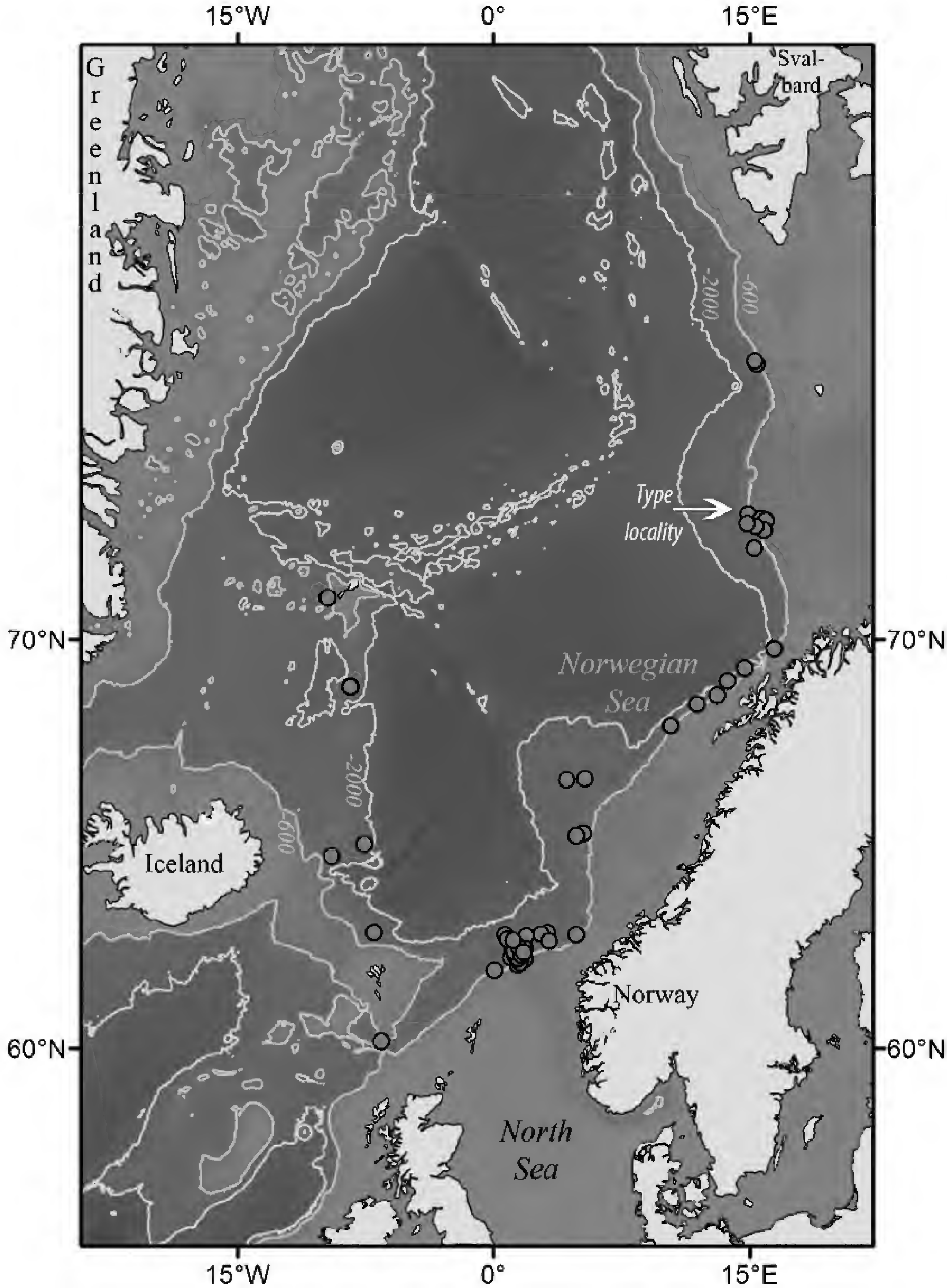

Figure 1. Map of the Nordic Seas showing type locality and records of *Ampharete undecima* sp. nov. Background map based on GEBCO08 and the Ocean Basemap (March 2013) by ESRI.

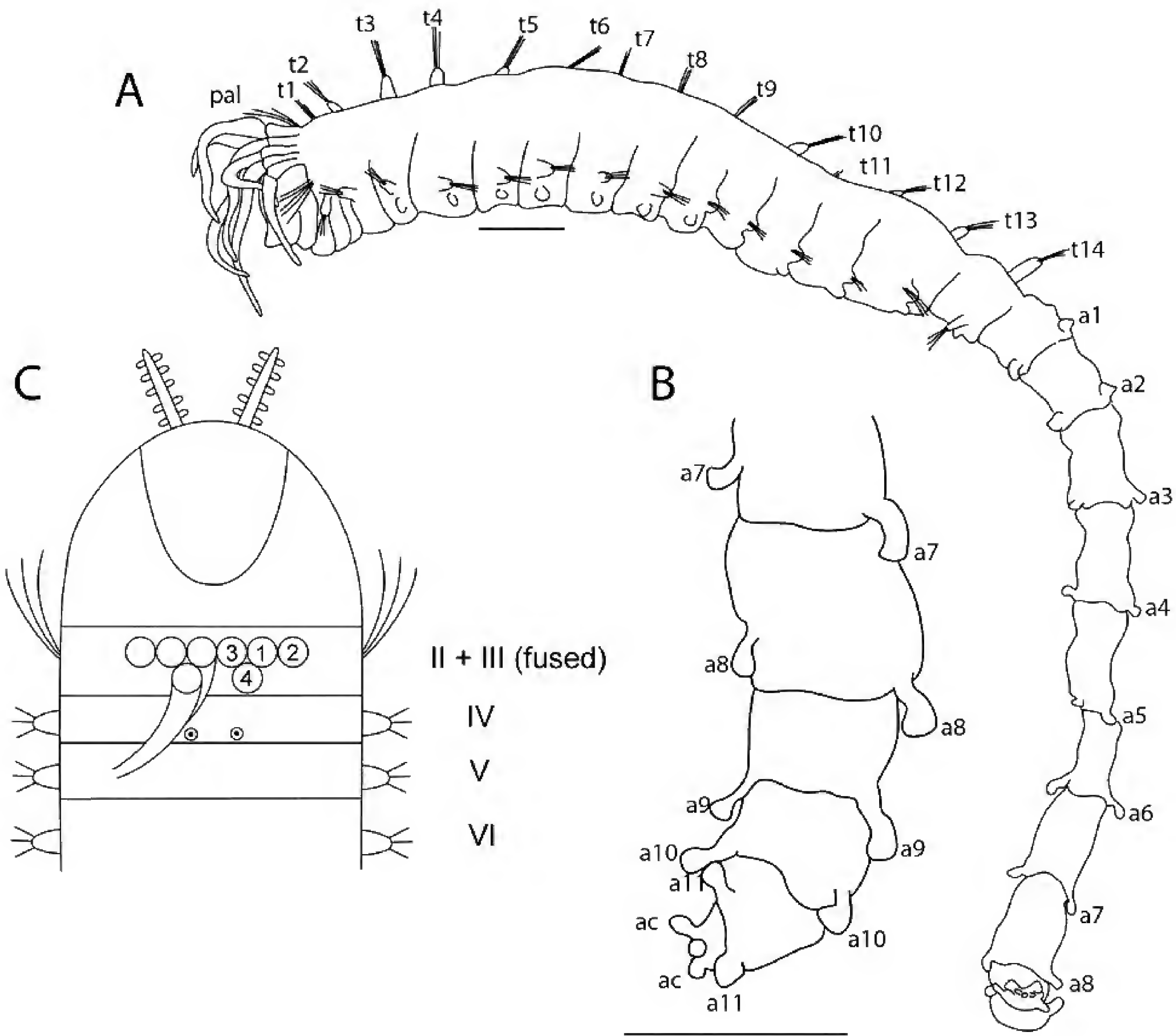

Figure 2. *Ampharete undecima* sp. nov. (A) Habitus of holotype (ZMBN 94022), dorsolateral view, posterior part of body twisted and the last 3 abdominal chaetigers are not distinguishable in drawing; (B) posterior end of holotype, ventral view; (C) schematic drawing of head and anterior end of body, indicating placement and origin of branchiae, and position of paired nephridial papillae on segment IV. Abbreviations: a1–11, abdominal chaetigers; ac, anal cirri; pal, paleae; t1–14, thoracic chaetigers. Scale bars: 250 μm.

III with 9–12 long, thin and slender paleae on each side, with curved tips (figs 3B, D, 5A). Thorax and abdomen of similar length; thorax slightly wider than abdomen, slightly tapering posteriorly (figs 2A, 3C). Abdomen of similar width throughout, or slightly tapering posteriorly. A total of 14 thoracic segments with notopodia and capillary chaetae. Last 12 chaetigers of thorax with neuropodia and uncini (figs 2A, 3A, C). Notopodia simple, finger-shaped; first 2 reduced, remaining 12 up to 3 times longer than wide. Notochaetae as spinulose capillaries (fig. 5F–G), arranged in double rows; capillaries from anterior row generally thinner and shorter than from posterior row. Thoracic neuropodia rounded to oval (fig. 3C). Thoracic uncini with two vertical rows of 4–6 teeth above rostrum (fig. 5B–C). Continuous ventral shields present to thoracic unciniger 8. A total of 11 abdominal unciniger (fig. 2A–B, 3C). Anterior 2 abdominal segments with neuropodia as thoracic type (fig. 3C); remaining abdominal uncinigers with enlarged neuropodia without cirri (figs 3A, C, 5D). Abdominal uncini with 4 vertical rows of 4–6 teeth above rostrum (fig. 5D–E). Pygidium with

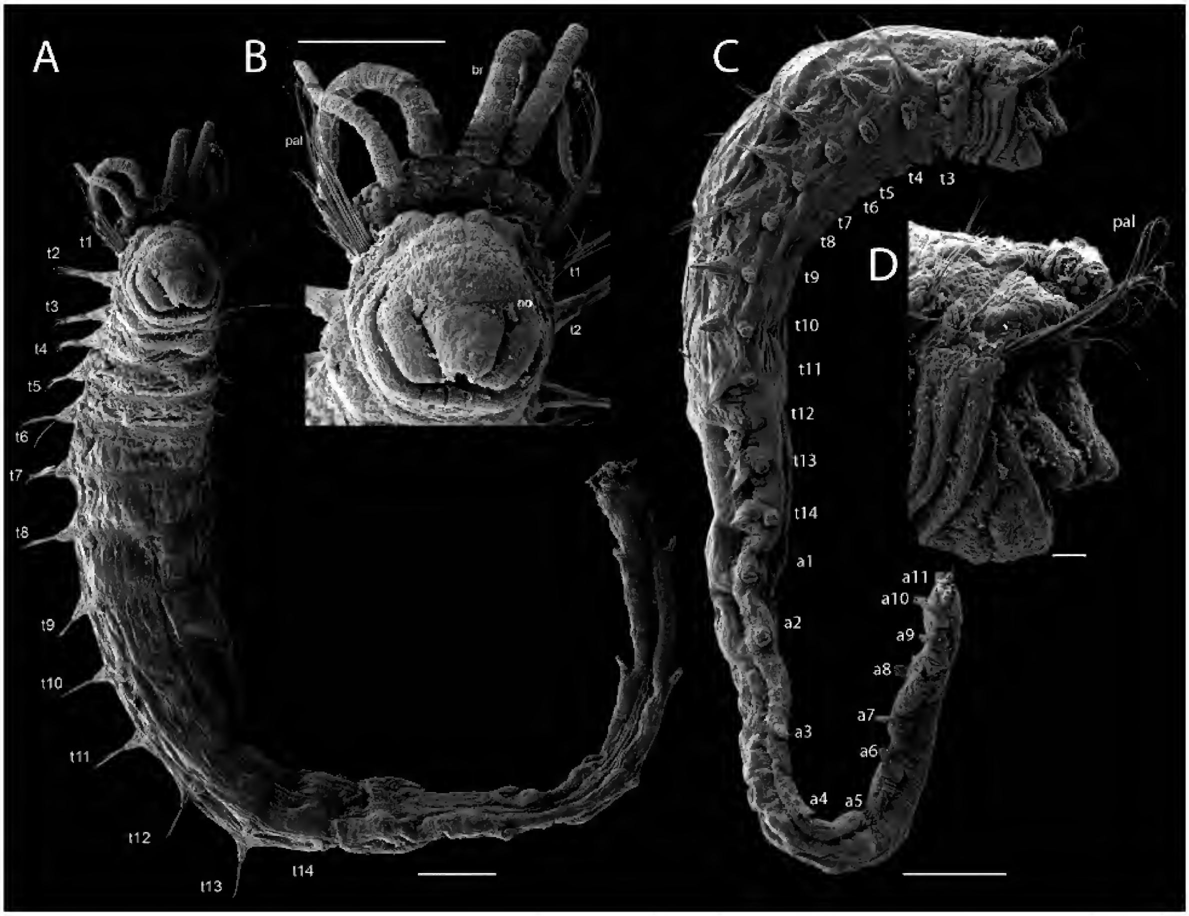

Figure 3. *Ampharete undecima* sp. nov. (A) Habitus, frontal and ventral view, posterior end of body missing; (B) head, frontal view, enlarged from A; (C) habitus, lateral view; (D) head, lateral view, enlarged from C. A–B, paratype (ZMBN 94023, spm #1); C–D, paratype (ZMBN 94023, spm #2). Abbreviations: a1–11, abdominal chaetigers; br, branchiae; no, nuchal organs; pal, paleae; t1–14, thoracic chaetigers. Scale bars: A, C, 200 μm; B, D, 20 μm.

terminal ciliated anal opening, surrounded by 2 short lateral cirri and small rounded papillae (figs 2B, 4D). Head and ventral shields dyed by methyl blue; anterior tip of prostomium with particularly strong colour (fig. 6A–C). Tube unknown.

Distribution. Common and widespread in the Nordic Seas in depths ranging from 600–1650 m (fig. 1).

Etymology. The species is named after the Latin word for eleven, referring to the eleven abdominal segments.

Remarks. Ampharete undecima sp. nov. is referred to the genus *Ampharete* based on the presence of a trilobed prostomium without glandular ridges, and with the median lobe delimited by deep grooves, buccal tentacles with pinnulae, the presence of four pairs of branchiae arising from the fused segment II+III, 12 thoracic uncinigers, and a single pair of nephridial papillae located dorsally on segment IV (Parapar et al., 2012; Imajima et al., 2012).

Ampharete undecima sp. nov. differs from all known species of *Ampharete* in having 11 rather than 12–28 abdominal uncinigerous segments. In the Norwegian Sea, *A. undecima* sp. nov. commonly occurs together with two other species of the genus, *A.* cf. *lindstroemi* (Malmgren, 1867) and *A. finmarcicha* (M. Sars, 1865) (Alvestad and Kongsrud, pers. obs.). *A. undecima* sp. nov. may easily be distinguished from both by a number of characters in addition to the number of abdominal uncinigers, including body size, the narrow and tapering middle lobe of the prostomium, number and shape of paleae, and arrangement of the branchiae (Holthe, 1986; Parapar et al., 2012; pers. obs.).

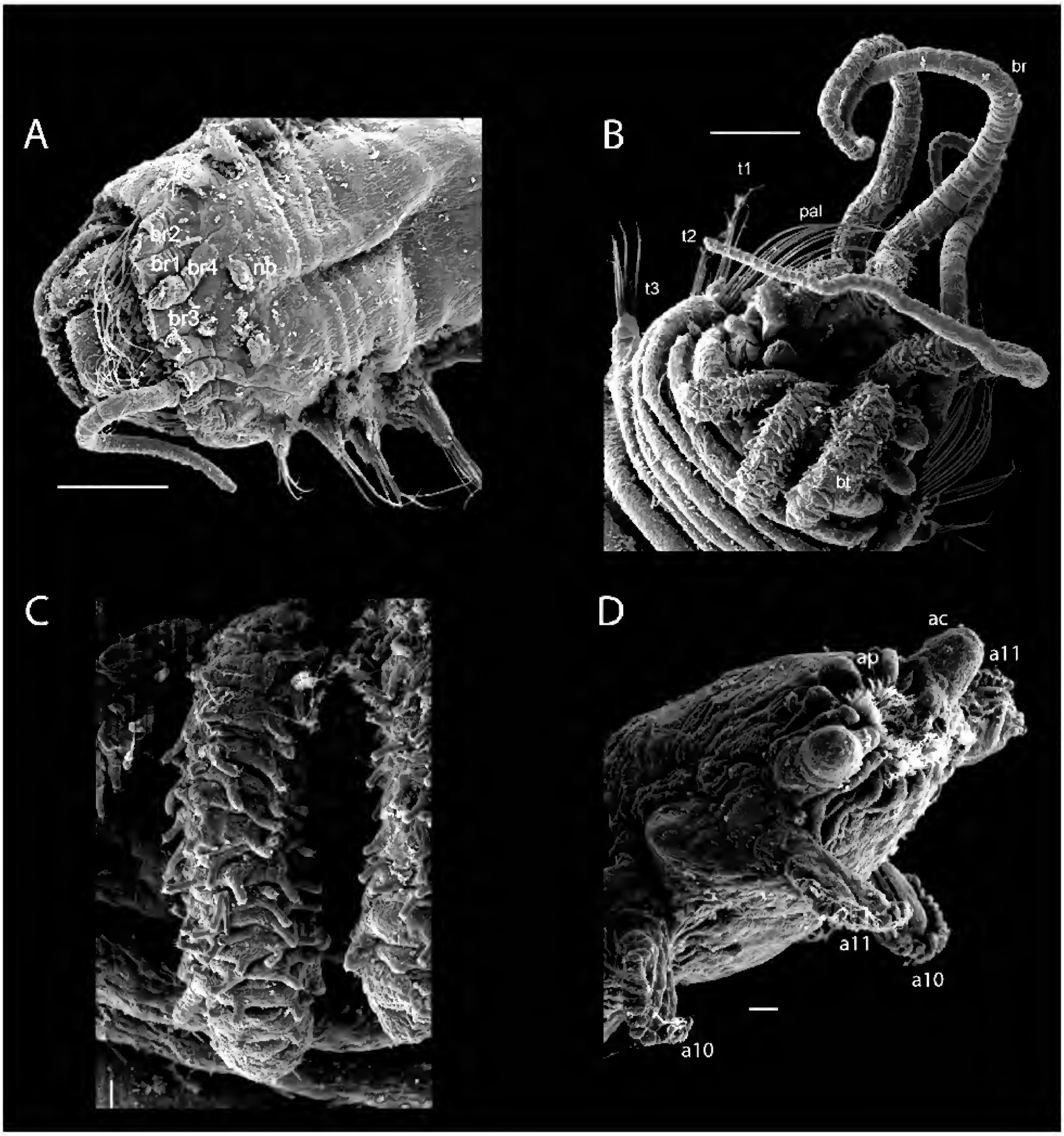

Figure 4. *Ampharete undecima* sp. nov. (A) Head and anterior part of body, dorsal view; (B) head, ventral view; (C) detail of buccal tentacle, enlarged from B; (D) posterior part of body and pygidium, lateral view. A, specimen from RV *H. Mosby* stn 84.05.23.1; B–C, specimen from RV *H. Mosby* stn 83.06.03.2; D, paratype (ZMBN 94023, spm #2). Abbreviations: a10–11, abdominal chaetigers 10–11; ac, anal cirri; ap, anal papillae; br1–4, branchiae; bt, buccal tentacles; np, nephridial papillae; pal, paleae; t1–3, thoracic chaetigers 1–3. Scale bars: 10 μm.

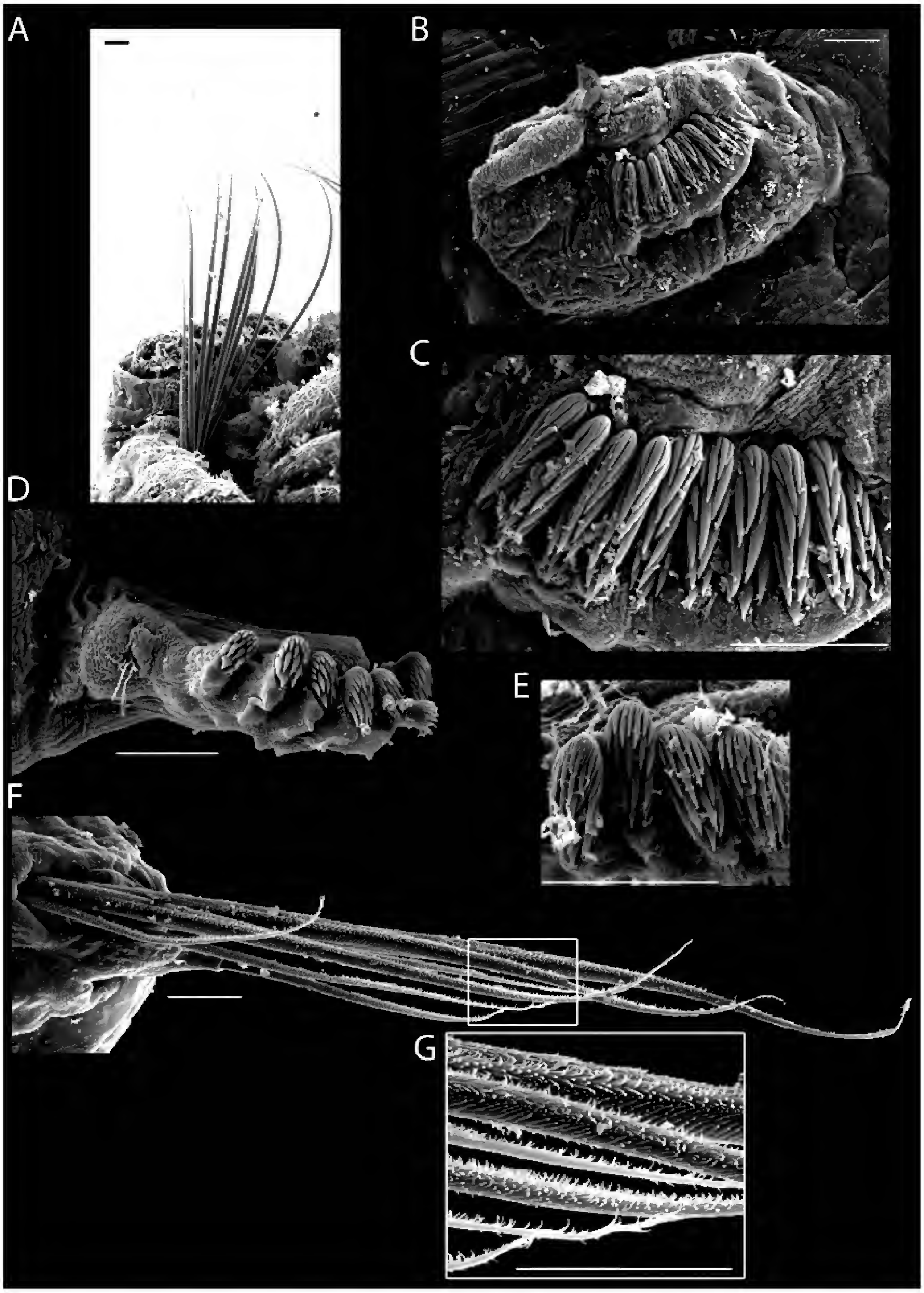

Figure 5. *Ampharete undecima* sp. nov. (A) Details of paleae; (B) thoracic uncini from chaetiger 5; (C) details of thoracic uncini, enlarged from B; (D) abdominal uncini from chaetiger 22; (E) details of abdominal uncini; (F) notopodium with capillary chaetae; (G) scale covering of capillary chaetae, enlarged from F. Scale bars: 10 μm.

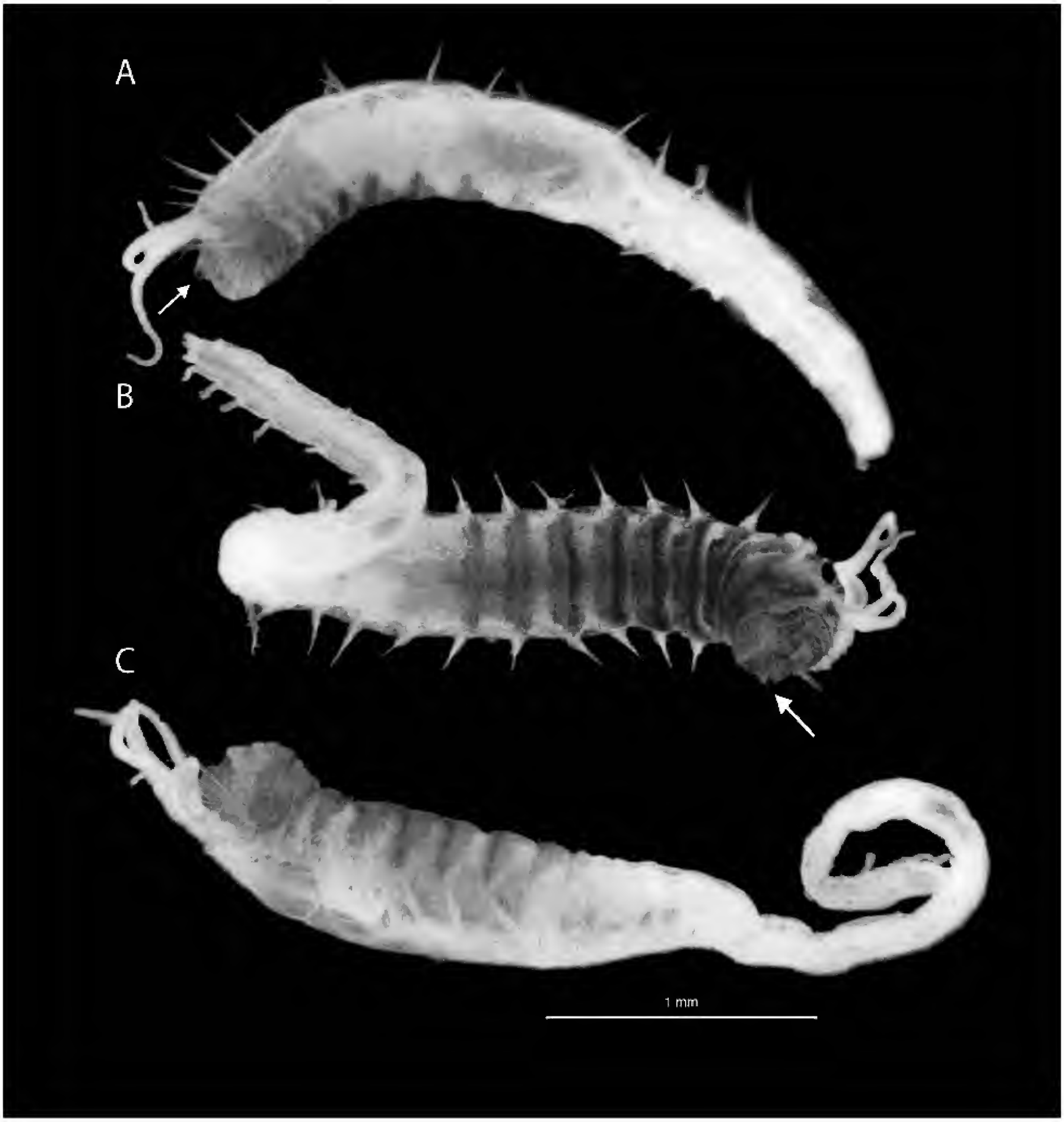

Figure 6. *Ampharete undecima* sp. nov. Paratypes (ZMBN 94024). Methyl blue staining pattern. (A, C) lateral, (B) ventral. Characteristic deep stain on the anterior tip of the prostomium indicated by arrows in figure.

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Do symbiotic polychaetes migrate from host to host?

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Abstract

Britayev, T.A. and Mekhova, E.S. 2014. Do symbiotic polychaetes migrate from host to host? *Memoirs of Museum Victoria* 71: 21–25.

It is generally considered that symbiotic animals colonise their hosts during their early stages of development. The main goal of the present study was to assess whether post-settled stages (juvenile and adult) of the symbiotic polychaete *Paradyte crinoidicola* are able to colonise their host comatulid crinoids. We also considered possible motives for symbiont migrations based on the intraspecific traumatism, size and sex structure data, and distribution pattern of *P. crinoidicola*. To this end, field sampling and experiments with depopulated hosts of the comatulid crinoid *Himerometra robustipinna* were carried out. The infestation prevalence was 62%, each infested host harbored from 1 to 7 polychaetes, and multiple infestations with 2 or 3 polychaetes per host were common. Mean intensity was 2.1 specimens per host. The dispersion coefficient was 1.7, greater than 1, indicating the tendency to contagious distribution pattern. Male/female ratio in *P. crinoidicola* was very close to the expected 1:1 ratio. About 33 % of *P. crinoidicola* had a traumatised posterior ends, and 31% damaged and regenerated parapodia, elytra and cirri, likely attributable to intra-specific fighting. In the field experiments depopulated crinoids were rapidly colonised by symbionts. The infestation characteristics of recolonised hosts didn't differ significantly to that of the control. Mean length of polychaetes and the ratio of small polychaetes to large polychaetes were similar in the experimental series and in the control, indicating a colonisation of crinoids not only by settling larvae, but predominately by migrating post-settled juveniles and adults. The male/female ratio deviated significantly in favor of males in the experimental series, suggesting that males more than females actively migrate among hosts. Intraspecific competition and searching for mating partners are proposed as causes for host swapping in *P. crinoidicola*.

Keywords Polychaeta, *Paradyte crinoidicola*, symbiont, traumas, sex ratio, migration, host switching, recolonisation, Comatulida, crinoids, Vietnam.

Introduction

Obligatory symbiotic animals are well adapted morphologically and behaviorally to live in close association with their hosts, while it seems likely that they are vulnerable to predators during movements between the hosts (Castro, 1978). Thus, it was considered that interactions among particular symbiotic associations are established only during close contacts between hosts, as in the case of the crab (*Liopetrolisthes mitra*) inhabited sea urchins (Thiel et al., 2003), or the polychaetes (*Histriobdella homari*) associated with lobsters (Simon, 1968). Nevertheless, host-to-host migrations suggest the existence of a free-living stage in the life-cycles of symbionts, which was already demonstrated in several species of crabs and ophiuroids (e.g. Castro, 1978; Fourgon et al., 2007; Bruyn et al., 2009). Host switching was also found in the crinoid-associated shrimp *Synalpheus stimpsoni* (VandenSpiegel et al., 1998) and in a few species of symbiotic polychaetes (Lande and Reish, 1968; Dimock, 1974; Britayev, 1991). It was supposed that host-to-host migrations should be a rather common phenomenon in symbionts with territorial behavior (Martin and Britayev, 1998). Motives for host swapping include searching for better shelter and food supply, mating partners, and intraspecific and interspecific competition (Castro, 1978; Thiel et al., 2003). However, it is not clear whether all these motives are relevant for each particular species, or if motives differ in different species.

To verify the existence of host-to-host migrations in symbiotic polychaetes we selected the scaleworm *Paradyte crinoidicola* (Potts, 1910) as it is one of the most common symbiotic polychaetes in tropical shallow waters with evidence of territoriality (Britayev et al., 1999). This species is widely

distributed in the Indo-West Pacific and inhabits more than 30 species of shallow-water unstalked crinoids or comatulids with relatively high (14 to 48%) infestation prevalence (Zmarzly, 1984; Britayev and Antokhina, 2012).

The main goal of the present study was to assess experimentally whether post-settled juvenile and adult *P. crinoidicola* migrate from host to host. We also considered intraspecific traumatism, size and sex structure, distribution pattern of polychaetes, and based on data obtained, possible motives for the migrations of symbionts.

Material and methods

Sampling of crinoids and their symbionts, and field experiments were carried out in the outer part of Nhatrang Bay (South China Sea, South Vietnam), near eastern coast of Tre Island.

Host crinoid *Himerometra robustipinna* (Carpenter, 1881) employed in our studies is common in the Bay of Nhatrang, where it forms dense aggregations up to 10–15 individuals per m^2. Individuals are usually bright-red colored, which easily distinguishes them from other crinoids *in situ* (fig. 1a). *P. crinoidicola* is very abundant in the area (fig. 1b) and inhabits all the comatulids found in the Bay.

Specimens of *H. robustipinna* were hand-collected by SCUBA diving at 6–10 m depth. Individuals were gently pulled away from the substrate, and immediately placed in separate zip-lock plastic bags to avoid loss of symbionts. On the boat, crinoids were carefully checked and all visible polychaete symbionts were removed and fixed in 70% alcohol. Later in the laboratory, polychaetes were measured, sexed, and traumas recorded. Although individuals easily fragmented, body measurements are possible due to high correlation between length and width ($y = 12{,}88x + 0{,}328$, where y = length, x = width, $R^2 = 0{,}983$). Thus, only body width between bases of parapodia of the widest segments was measured. Sex was determined by the presence of oocytes in females, spermatids or spermatozoa in males. For that purpose, 1–2 midbody segments were placed on a slide in a drop of glycerol, covered by a coverslip, and analysed with a light microscope. Traumas to body and parapodia were recorded according to Britayev and Zamishliak (1996). Two main types of traumas were distinguished: small traumas (i.e. damaged elytra, cirri or parapodia, either lacking or being smaller than those of nearby segments as a consequence of regeneration processes, probably attributable to intra-specific aggressive behavior) (fig. 2b, d) and large traumas (primarily posterior end of body lost and regenerated, probably as a result of predators, e.g. fish and crustacean attacks) (fig. 2 c). Specimens lacking elytra, cirri, and posterior body end without traces of regeneration were not considered as traumatised.

To characterise the infestation of *H. robustipinna* by *P. crinoidicola* we determined the proportion of crinoids infested (prevalence) and the mean number of symbiont individuals per host infested (mean intensity). To determine the significance of differences in the male/female ratio and prevalence we used φ- test - angular Fisher transformation. To determine the significance of differences in the mean intensity and mean length we used t-test. To assess the distribution of polychaetes among hosts we employed the ratio of variance (σ^2) to mean value (μ), σ^2/μ (coefficient of dispersion). If a population has a random distribution, this ratio is close to 1.0. If the population distribution is more uniform than random $\sigma^2/\mu < 1.0$, and if the population is distributed contagiously, $\sigma^2/\mu > 1.0$ (Zar, 1984).

For field experiments the area characterised by the presence of large boulders and rocky outcrops, which are suitable substrates for crinoids were selected. These boulders were separated from each other by coarse sand with dead shells and pebbles, which in general is an inappropriate substrate for crinoids.

The study design included three experimental series and a control. In the first two series depopulated and tagged specimens of *H. robustipinna* were placed on boulders with dense aggregation of crinoids to test whether post-settled symbionts are able to migrate among host individuals within the locality. In the third series a group of depopulated hosts was placed on the isolated boulder without further crinoids to test the influence of spatial isolation on host colonisation by symbionts.

A total of 42 crinoid individuals collected together with their symbionts served as control. In each of the three series of experiments 14 depopulated hosts were used. After a one-week exposure, all experimental hosts were collected and analysed. Crinoids were carefully checked for symbionts and symbionts themselves were processed as described above. It was assumed that all *P. crinoidicola* exceeding 5 mm in length or with developed sexual reproductive structures infesting depopulated crinoids were the result of migration events. This takes into account the size of late nectochaetae (Bhaud and Cazaux, 1987) and a few observations on the growth of post-settled scaleworms (Pernet, 2000). More details on the experimental design and area studied are described in a general paper dedicated to recolonisation of *H. robustipinna* by associated symbiotic community (Dgebuadze et al., 2012).

Results.

Infestation characteristics and traumatism in the control.

From the 42 crinoid specimens employed in the experiments we found 26 infested with *P. crinoidicola*, i.e. prevalence of 62%. Altogether 54 polychaetes were found, most (88.9%) with gametocytes in the body cavity. The mean length of the polychaetes was 9.3 mm. The ratio of small (L 4–7 mm) to large polychaetes (L 8–15 mm) was 0.3:1. Each infested host harboured from 1 to 7 polychaetes, and multiple infestations with 2 or 3 polychaetes per host were very common (Table 1). The mean intensity was 2.1 specimens per host. The dispersion coefficient was 1.7 (Table 1). Male/female ratio in *P. crinoidicola* was very close to the expected 1:1 (chi-square 0.083, $P > 0.1$).

Among *P. crinoidicola* infesting *H. robustpinna*, 33% showed “large” traumas. The proportion of animals with damaged and regenerated parapodia, elytra and cirri (small traumas) was similar with 30% (Table 1).

Recolonisation experiments

After 7 days of exposure all tagged crinoids except one from series 3 were recorded. The data revealed that depopulated crinoids were rapidly colonised by symbionts. The prevalence

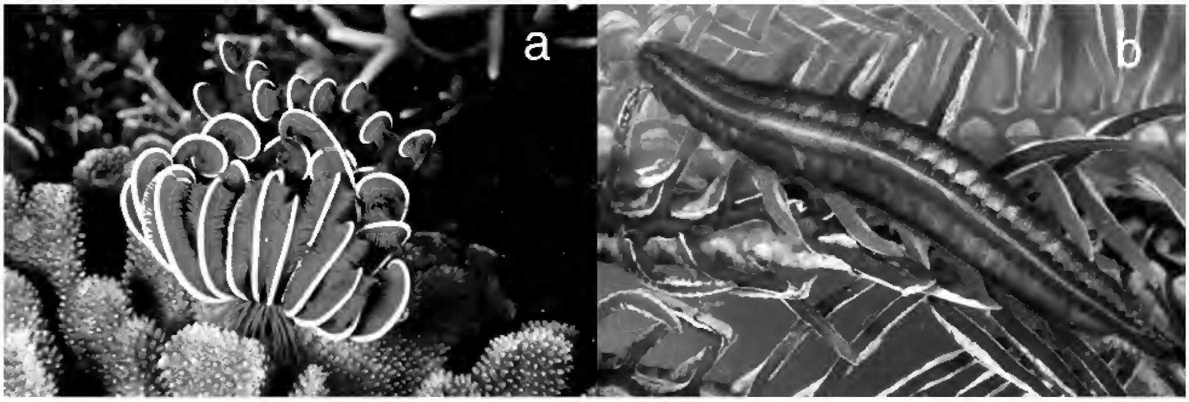

Figure 1. a. Host crinoid *Himerometra robustipinna* (Carpenter, 1881) *in situ*. b. Polychaete *Paradyte crinoidicola* (Potts, 1910) on the arm of the host.

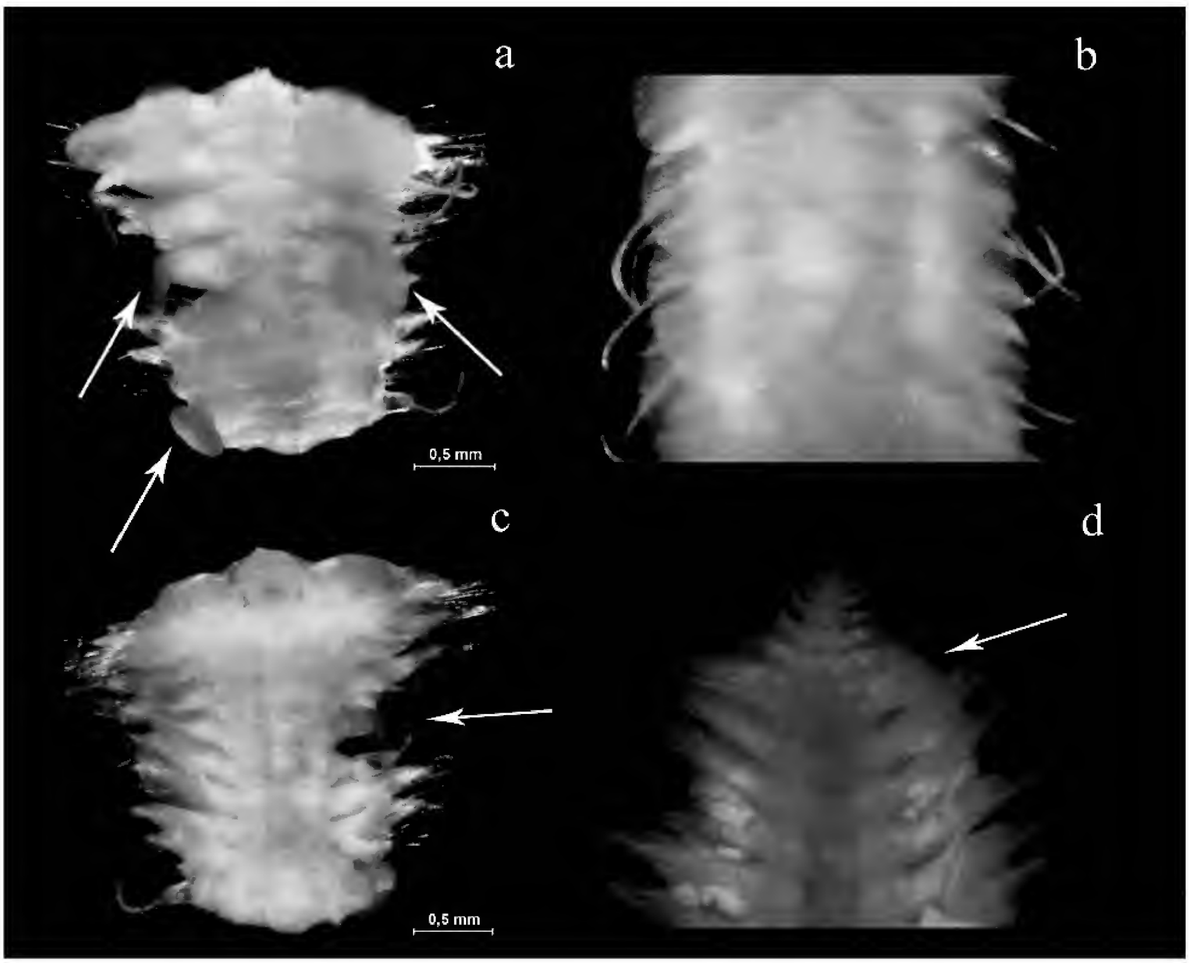

Figure 2. Traumas of *Paradyte crinoidicola*. a. Traumatized and regenerated elytra (white arrows). b. Dorsal surface of *P. crinoidicola* covered by unaffected elytra. c. Damaged and partially regenerated parapodium (white arrow). d. Traumatized and regenerated posterior end of body. White arrow indicates border between old and new chaetigers.

Table 1. Infestation characteristics, size, male/female ratio, traumas of *Paradyte crinoidicola*, and number of hosts (in parenthesis) in the control and in the experimental series.

Indices	Control (42)	Series 1 (14)	Series 2 (14)	Series 3 (13)
Symbionts number	54	13	10	24
Prevalence (infested hosts)	62% (26)	57% (8)	50% (7)	64% (8)
Mean intensity (±SD)	2.1 (±1.4)	1.6 (±1.1)	1.4 (±0.5)	2.7 (±1.7)
Mean length. mm (±SD)	9.3 (±2.9)	10.2 (±3)	8.9 (±2.1)	8.7 (±3.3)
Small/large worm ratio	0.3	0.2	0.2	0.3
Dispersion coefficient	1.7	1.1	0.3	4.3
Male/female ratio	1.1	1.4	3.5	5.7
Small traumas (%)	30	15	0	42
Large traumas (%)	33	38	60	29

of infestation was high and close to that in the control, while mean intensity of recolonised hosts deviated in both sides to that of the control (Table 1). The dispersion coefficient varied significantly from 0.3 in series 2 to 4.3 in series 3. This variability correlates rather with the low number of polychaetes in series 1 and 2 than with biological interactions.

Mean length of polychaetes and the ratio of small to large polychaetes were similar in the experimental series and in the control (Table 1). Male/female ratio deviated in favour of males in the experimental series (joint samples, chi-square 10.8, $P < 0.01$). This deviation was insignificant in series 1 and 2 (chi-square 0.3 and 2.8 respectively, $P > 0.1$), but increased substantially in the spatially isolated locations in series 3 (Table 1, chi-square 9.8, $P < 0.01$). The proportion of traumatised polychaetes varied for specimens with small traumas from 0 to 42%, and for specimens with large traumas from 29 to 60 (Table 1).

Discussion

Paradyte crinoidicola are very fragile animals, which easily fragment when disturbed and lose elytra and cirri, both original and regenerated. Thus, the actual number of animals with both types of traumas should be higher than observed, and for this particular species of scaleworm traumas of parapodia are the most relevant mark of intraspecific interactions. The high frequency of traumas similar to that in other symbiotic scaleworms with intraspecific competition for the host territory, *viz*. *Arctonoe vittata*, *Gastrolepidia clavigera*, *Branchipolynoe seepensis* (Britayev, 1991; Britayev and Zamyshliak, 1996; Britayev et al., 2007), suggests territoriality also in *P. crinoidicola*. On the other hand, our observations on host infestation and tendency to contagious distribution of polychaetes among hosts (the dispersion coefficient higher than 1 in the control and series 3, Table 1), disagree with the expected regular distribution in species with territorial behavior (*e.g.* Odum, 1971) and data on solitary distribution of *P. crinoidicola* among comatulid hosts in the Red Sea (Fishelson, 1985). We suggest, that this particular situation, *viz*. co-occurrence of contagious distribution and territoriality, is related to several circumstances: (1) tolerance of adult and juvenile residents to recruits, already known in some other symbionts with territorial behavior, *e.g.* the crab *Allopetrolisthes spinifrons* (Baeza et al., 2002), (2) relatively large size of the host comatulid *H. robustipinna* and (3) its morphological complexity, providing isolated microhabitats for polychaetes. The discrepancy to Fishelson's observations is probably related to predation pressure regulating the abundance of polychaetes, which is low due to overfishing in the Bay of Nhatrang and relatively high in the Red Sea area studied by Fishelson (senior author's personal observation).

Our data revealed that depopulated crinoids were rapidly colonised by symbionts. The infestation characteristics of recolonised hosts didn't differ significantly to that of the control. To determine whether polychaetes infest host by migration of already settled juveniles and adults from neighbouring comatulids, or by settlement of larvae from the plankton, the mean length of polychaetes and proportion of adults in the control and in the experimental series were compared. Similar means of both indices support the hypothesis on migration of polychaetes between hosts.

Presently, host-swapping behavior has been documented in only 3 polychaete species, the hesionid *Ophiodromus puggetensis* (Lande and Reish, 1968), and the scaleworms *Arctonoe pulchra* and *A. vittata* (Dimock, 1974; Britayev, 1991). With *P. crinoidicola* our experiments revealed one further species with such a behavioral adaptation, suggesting it is a more common phenomenon among symbiotic polychaetes than has been considered so far, and proved indirectly a link between territoriality and host-to-host migrations (Britayev, 1991).

The experimental series 1 and 2 indicated movement of symbionts in dense host aggregations or over short distances. It has been suggested such migrations are more common between hosts with contagious distribution patterns than between spatially dispersed hosts (Thiel et al., 2003). The infestation characteristics of crinoids in the spatially isolated site (series 3) were not lower than that of crinoids from aggregations of series 1 and 2, suggesting also extensive long-distance host-to-host migrations. This unexpected result

indicates the ability of symbionts to rapidly cross inappropriate biotopes and requires special consideration.

As has been demonstrated earlier (Britayev, 1991), intraspecific aggressive interactions accompanied by traumas of body appendages lead to relocation of symbiotic polychaetes from one host to another. The high frequency of traumas in the control and series 1 and 3 indirectly indicates intraspecific interactions in *P. crinoidicola* populations, so we can suggest that one reason of their host-to-host migration is intraspecific competition. Another possible reason likely is the search for mating partners. The deviation in the sex ratio in favor of males in experimental series indicates a higher migratory activity of males in comparison to females. This latter phenomenon attributed to searching for a mate is well known in symbiotic crabs (*e.g.* Wirtz and Diesel, 1983; Yanagisawa and Hamaishi, 1986; Baeza, 1999; Thiel et al., 2003), but only recently documented in symbiotic polychaetes (Britayev et al., 2007).

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New symbiotic associations involving polynoids (Polychaeta, Polynoidae) from Atlantic waters, with redescriptions of *Parahololepidella greeffi* (Augener, 1918) and *Gorgoniapolynoe caeciliae* (Fauvel, 1913)

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Abstract

Britayev, T.A., Gil, J., Altuna, Á., Calvo, M. and Martín, D. 2014. New symbiotic associations involving polynoids (Polychaeta, Polynoidae) from Atlantic waters, with redescriptions of *Parahololepidella greeffi* (Augener, 1918) and *Gorgoniapolynoe caeciliae* (Fauvel, 1913). *Memoirs of Museum Victoria* 71: 27–43.

Different circumstances such as sampling methodology, sample sorting or taxa distribution among different experts often lead symbiotic associations to remain hidden and the mode of life of the involved partners are either not defined or directly reported as free living. This was apparently the case of *Parahololepidella*, a genus proposed by Pettibone (1969) to include *Hololepidella greeffi* Augener, 1918, reported as free-living from shallow waters off São Tomé and Cabo Verde Islands (W Africa). In this paper, we report for the first time the symbiotic status of *P. greeffi* (Augener, 1918), which lives in association with the antipatharian *Tanacetipathes* cf. *spinescens* (Gray, 1857), based on new materials collected in São Tomé Island. In addition to the originally described features, the species is characterized by a variable presence of cephalic peaks and by an irregular distribution of elytra from segment 32-33, which may be asymmetrical (within the same individual) and differ between individuals. A list of all known polychaete species associated with antipatharian corals is also provided. We also report new findings of *Gorgoniapolynoe caeciliae* (Fauvel, 1913) from deep waters of the Atlantic coasts of the Iberian Peninsula, living in association with the octocorals *Candidella imbricata* (Johnson, 1862) (first report for the Spanish waters) and *Corallium niobe* Bayer, 1964. The diagnosis of *Gorgoniapolynoe* is emended and we suggest that *G. corralophila* (Day, 1960) should be referred to a different genus and that *G. pelagica* Pettibone, 1991a should be considered as *nomen dubium*. The Iberian *G. caeciliae* fits well with the re-description by Pettibone (1991a), except for the presence of clavate papillae on dorsal cirri, which were neither mentioned nor figured in previous descriptions.

Keywords New symbiotic associations; Polynoidae; Myriopathidae; Primnoidae; Coralliidae; São Tomé Island; Cabo Verde Island; Iberian Peninsula.

Introduction

Among the polychaete families, the Polynoidae includes the highest number of symbiotic species. There were about 163 species involved in more than 420 relationships reported by Martin & Britayev (1998), but the number has increased continuously since then and currently exceeds 200 species involved in about 550 relationships (D. Martin, unpublished data).

Different circumstances (such as sampling methodology, sample sorting, or taxa distribution among the different experts) often lead symbiotic associations to remain hidden. Consequently, the mode of life of the involved partners is either not defined or directly reported as free living. Some new reports may correspond to these "hidden" associations, which turned to be recognized as symbiotic when new or more precise observations were carried out. This is the case for *Parahololepidella*, a genus proposed by Pettibone (1969) to include *Hololepidella greeffi* Augener, 1918. All known specimens of this species were reported as free-living from shallow waters off São Tomé and Cabo Verde Islands (Augener, 1918; Pettibone, 1969).

New specimens of this species were found among newly collected materials from São Tomé Island, housed and sorted in the Museo Nacional de Ciencias Naturales (MNCN-CSIC) of Madrid, and from Cabo Verde Island (collected during an expedition to the Canarias – Cape Verdian region, CANCAP), housed and sorted in the Naturalis - Nationaal Natuurhistorisch Museum, Leiden (NNMN). All newly collected specimens were living in association with the antipatharian *Tanacetipathes* cf. *spinescens* (Gray, 1857) (Myriopathidae). Consequently, we first report here the symbiotic status for *Parahololepidella greeffi* (Augener, 1918). Moreover, as some morphological details were not properly described in the original description, we provide a full re-description of the species, including some considerations on the status of *Hololepidella fagei* Rullier, 1964. A list of all known polychaete species associated with antipatharian corals is also provided.

Furthermore, we also report on new findings of *Gorgoniapolynoe caeciliae* (Fauvel, 1913) from deep waters off the Atlantic coasts of the Iberian Peninsula, living in association with the octocorals *Candidella imbricata* (Johnson, 1862) and *Corallium niobe* Bayer, 1964. Both the genus and the species are re-described based on these newly collected materials.

Material and methods

The specimens of *P. greeffi* and its host antipatharian *Tanacetipathes* cf. *spinescens* were collected in different locations off São Tomé and Cabo Verde Islands (see the corresponding Examined Material section and Table 1 for a detailed list of samples and locations). Specimens from São Tomé Island were directly fixed and preserved in 70% ethanol, while those from Cabo Verde were fixed in formaldehyde (10% in seawater) and later rinsed with fresh water and transferred to 70% ethanol.

The specimens of *G. caeciliae* were collected during the 2010 and 2011 expeditions of the INDEMARES project by the Spanish Institute of Oceanography (IEO), at the Galicia Bank (associated with *Candidella imbricata*) and at the Avilés Canyon System (associated with *Corallium niobe*). Samples were collected with the help of a hard bottom grab ("Draga de roca" in Spanish, DR in the respective sample codes). They were also directly fixed and preserved in 70% ethanol. Voucher specimens are deposited at the IEO (Gijón Laboratory, Spain) and the Okendo Museum (Donostia-San Sebastián, Spain).

Light microscope micrographs of relevant morphological characters were made at the Laboratory of Microscopy and Digital Photography of the CEAB, with the help of a ProgRes C10 Plus digital camera (Jenoptics, Jena) attached to a Zeiss Axioplan compound microscope (body) and a CT5 digital camera (Jenoptics, Jena) attached to a SMZ1000 Nikon stereomicroscope (parapodia). Drawings of parapodia were made using an Olympus U-DA *camera lucida* attached to an Olympus BX-41 microscope.

Abbreviations in text: af: anterior fragment; pf: posterior fragment; L: length; WW: width without parapodia and without chaetae; WC: width with parapodia and chaetae.

Taxonomic account

Family **Polynoidae** Kinberg, 1856

Subfamily **Polynoinae** Kinberg, 1856

Genus ***Parahololepidella*** Pettibone, 1969

Type species. Hololepidella greeffi Augener, 1918.

Diagnosis. Body long, slender, flattened, with sides nearly parallel, tapered posteriorly, with numerous segments (up to 140 or more). Elytra numerous up to 50 and more pairs, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, 32, thereafter irregularly arranged on alternate segments, often asymmetrical, with different number on right and left sides. Elytra oval smooth, without tubercles and micropapillae; first pairs medium sized, usually covering mid-dorsum; following ones very small, leaving mid-dorsum and parapodia uncovered. Prostomium bilobed, subtriangular anteriorly, with or without distinct cephalic peaks, with two palps and three antennae. Ceratophore of median antenna in anterior notch; lateral antennae inserted ventrally. First (tentacular) segment with a pair of tentaculophores inserted laterally to prostomium, with 1-2 aciculae and one slightly serrated unidentate notochaeta; facial tubercle prominent; mouth surrounded by two lateral lips, one dorsal with six–seven lobes, and one large ventral lip with 7-9 lobes. Second (buccal) segment with first pair of elytra, sub-biramous parapodia and long, tapering ventral cirri; without nuchal fold. Parapodia sub-biramous. Notopodia small, digitiform; notochaetae short, stout (not as stout as neurochaetae), tapering to blunt tips, unidentate. Neuropodia with longer rounded prechaetal lobes with subacicular digitiform processes; postchaetal lobes short, rounded; neurochaetae stout, with faint spinous regions, and slightly hooked, unidentate all of same type. Dorsal cirri smooth, with cylindrical, relatively long cirrophores and very long styles. Dorsal tubercles absent. Ventral cirri short, tapering. Nephridial papillae short, bulbous.

Parahololepidella greeffi (Augener, 1918)

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(Figs. 1-7)

Hololepidella greeffi: Augener (1918), p. 148, pl. 2, figs. 22-24, pl. 3, fig. 52, text-fig. 9; Hartman (1959), p. 81; Rullier (1964), p. 130, fig. 3.

Hololepidella fagei: Rullier (1964), p. 132, fig. 4; Hartman (1965), p. 9.

Parahololepidella greeffi: Pettibone (1969), p. 54–55, fig. 4; Kirkegaard (1983), p 192.

Material examined. Cabo Verde Archipelago. Santiago Island, SW coast near Ponta da Cidade, 14°54'N 23°38'W. Sta. CANCAP 7.D01, depth to 22 m, one specimen af and pf (NNMN 24481) on *Tanacetipathes* cf. *spinescens*, loose boulders on coarse sand, scuba diving, 20-21.08.1986, "Tydeman" Cabo Verde Islands Exped., 1986. W of Boa Vista Island, W of Ilhéu de Sal Rei, 16°11'N 23°00'W, Sta. CANCAP 7.081, depth 70 m, one complete specimen (NNMN 24644) on *Tanacetipathes* cf. *spinescens*, antipatharians and sponges, 1.2 m Agassiz trawl, 28.08.1986, "Tydeman" Cabo Verde Islands Exped., 1986.

Table 1. List of samples collected during the RSTP Cruise (2006) where *Parahololepidella greeffi* occurred in association with *Tanacetipathes* cf. *spinescens*. N: Number of worms per sample; WT: Water temperature (°C); Depth (m); fr: fragment.

Date	MNCN Catalogue Reference	N	Island	Station	Coordinates	WT	Depth
14/01/06	16.01/13707	8	São Tomé Is.	Lago Azul 2	00°24´19.0" N 06°36´26.6" E	27	20-25
15/01/06	16.01/13708	4	São Tomé Is.	Diogo Vaz 2	00°18´97.1" N 06°30´23.3" E	28	5-15
15/01/06	16.01/13709	1	São Tomé Is.	Diogo Vaz 1	00°18´53.2" N 06°29´23.3" E	27	20-25
18/01/06	16.01/13704	1	São Tomé Is. - Rolas Is.	Pedra do Braga	00°00´57.94"N 06°30´52.03"E	28	15-20
18/01/06	16.01/13706	1fr	São Tomé Is. - Rolas Is.	Pedra do Braga	00°00´57.94"N 06°30´52.03"E	28	15-20
18/01/06	16.01/13705	1	São Tomé Is. - Rolas Is.	Pedra do Braga	00°00´57.94"N 06°30´52.03"E	28	15-20

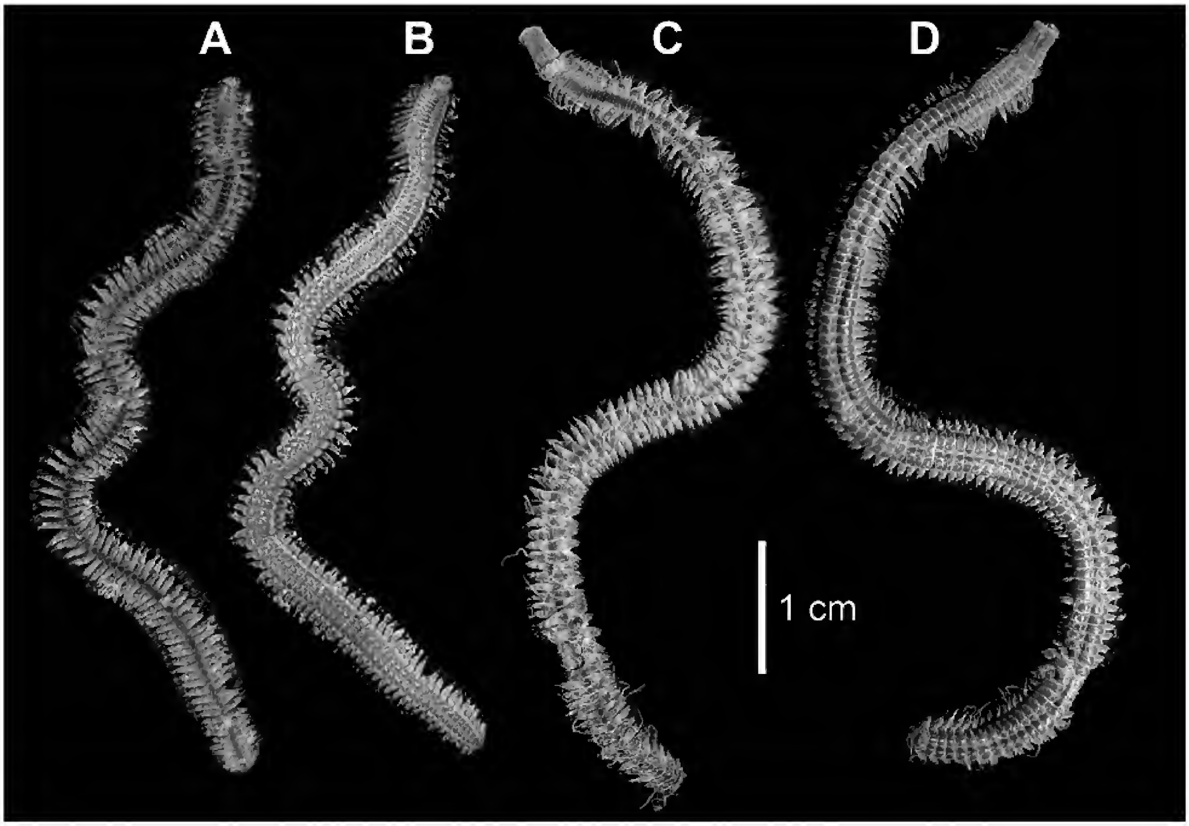

Figure 1.- *Parahololepidella greeffi*. MNCN 16.01/13708. (A, B) and MNCN 16.01/14341 (C, D). Adults in dorsal (A, C) and ventral (B, D) view.

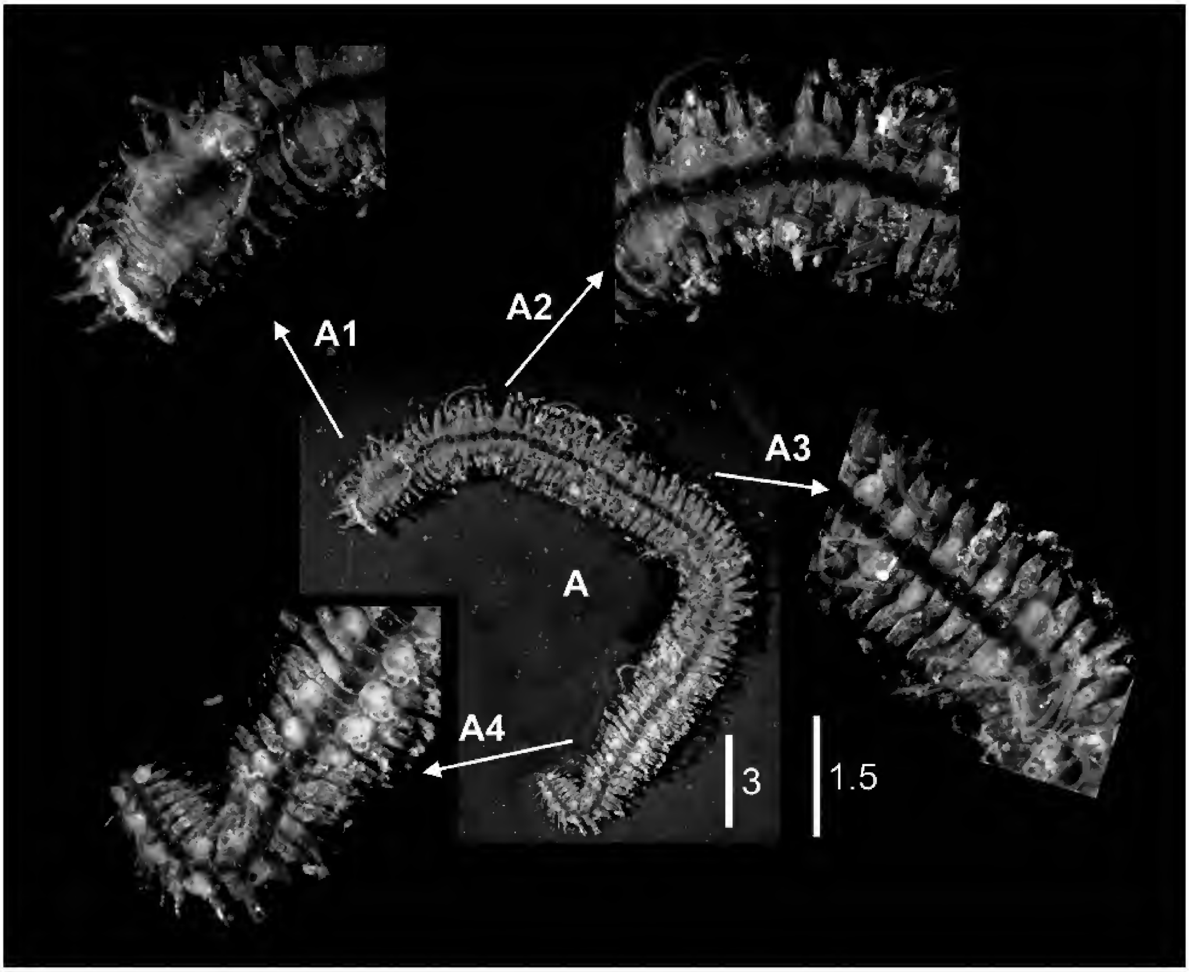

Figure 2.- *Paraholoepidella greeffi*. MNCN 16.01/13708. Juvenile. A. Entire view. A1 – A4. Detail of the anterior end (A1), mid-anterior region (A2), mid-posterior region (A3), and posterior end (A4). Scale bars are cm.

São Tomé e Príncipe Archipelago. 1 syntype, Ilha das Rolas, Zoological Museum of Hamburg (ZMH 5692); 16 worms (plus some fragments) on *Tanacetipathes* cf. *spinescens*, collected during the Republic of São Tomé e Príncipe (RSTP) cruise by CPD Service Supporting Science Research (Table 1).

Description. Based mainly on a well-preserved specimen, broken in two fragments, NNMN 24481). Body long, slender, dorso-ventrally flattened, with sides nearly parallel, tapering posteriorly, with up to 140 or more segments (figs. 1, 2). Without dorsal ciliary bands.

Prostomium slightly wider than long; cephalic peaks present or absent; ceratophore of median antenna in anterior notch, style smooth, tapering, longer than palps; lateral antennae inserted ventrally to median antenna, styles smooth, tapering; anterior pair of eyes dorso-lateral on widest part of prostomium, posterior pair dorsal, near posterior prostomial margin, slightly smaller than anterior ones; palps tapering. Facial tubercle prominent; mouth surrounded by two lateral lips, one dorsal with 6-7 lobes, and one large ventral lip with 7-9 lobes. Pharynx with four light-brown jaws, all similar in shape and size; nine pairs of large marginal pharyngeal papillae.

First (tentacular) segment with a pair of tentaculophores inserted laterally to prostomium, with one, rarely two aciculae and one slightly serrated unidentate notochaeta, with dorsal and ventral tentacular cirri, styles smooth, tapering. Second (buccal) segment with first pair of elytra, sub-biramous parapodia and long, tapering ventral cirri. Nuchal fold absent. Following segments with ventral cirri short, not reaching to tip of neuropodium. Cirrigerous segments without dorsal tubercle. Dorsal cirri smooth, with cylindrical, relatively long cirrophores and very long styles.

Elytra numerous up to 50 and more pairs, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, 32, thereafter irregularly

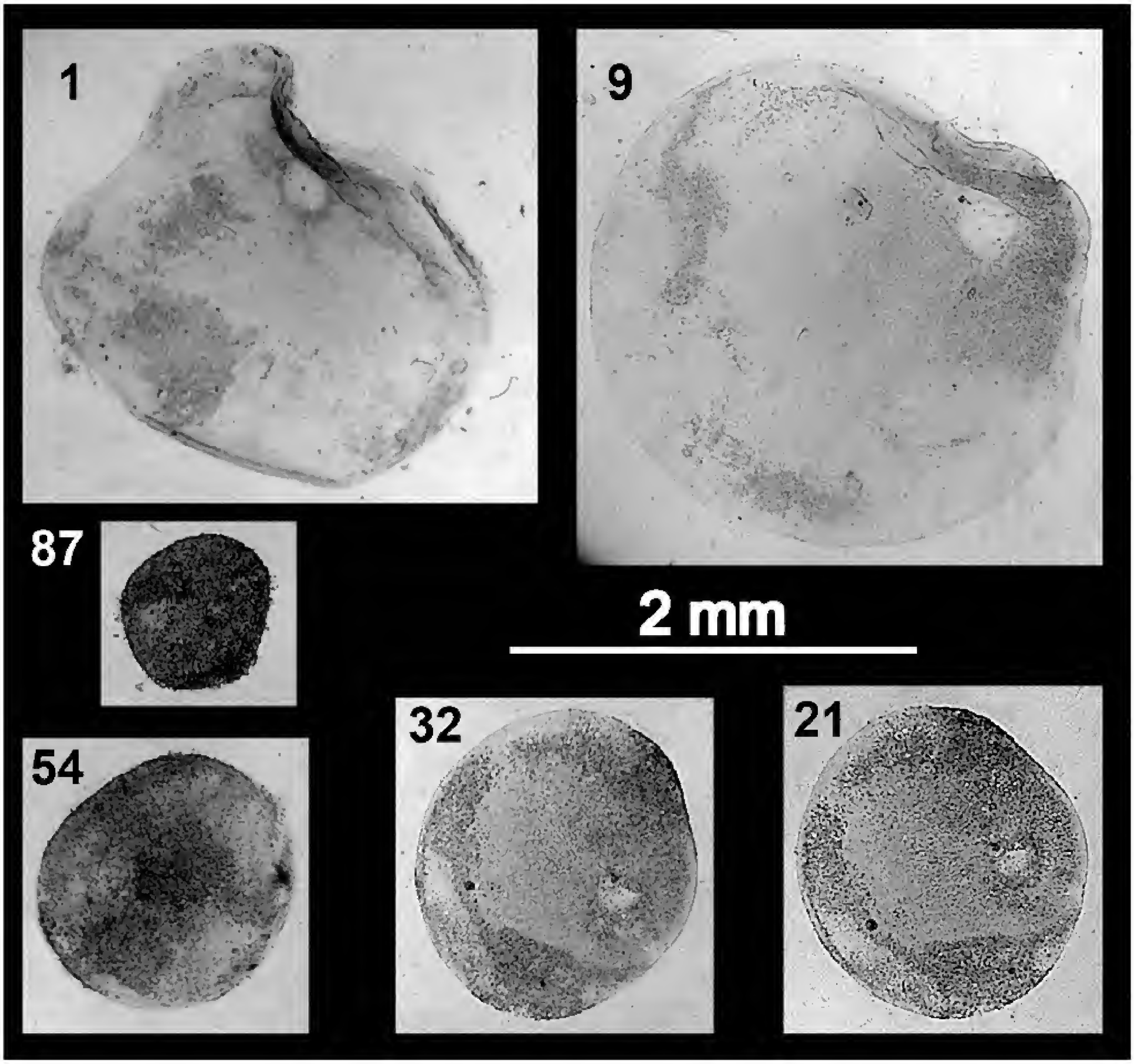

Figure 3.- *Parahololepidella greeffi*. MNCN 16.01/13708. Elytrae. Numbers represent the segment from which elytra were removed.

arranged on alternate segments, often asymmetrical, with different number on right and left sides (Table 2). Elytra almost oval in outline, smooth, soft, tubercles and micropapillae absent; first 11-12 pairs slightly folded, medium sized, usually covering mid-dorsum; following ones, very small, leaving mid-dorsum and parapodia uncovered (fig. 3).

Parapodia sub-biramous (fig. 4A). Notopodia small, digitiform (fig. 4B). Neuropodia with longer rounded prechaetal lobes with subacicular digitiform acicular lobe; postchaetal lobes shorter, distally rounded; tips of noto- and neuroacicula penetrating epidermis (figs. 4B-4D, 5A, 5B). Nephridial papillae short, bulbous, starting on segment 6 (fig. 4E).

Notochaetae slightly thinner than neurochaetae, few in number (0-5), nearly smooth, unidentate; neurochaetae few in number, but more numerous (5–10) than notochaetae, with unidentate tips and faint serration, all of same type (fig. 4F, 4G).

Surface of elytra and body often covered with scattered, angular, extraneous particles.

Measurements. 75-120 chaetigers, L 26-44 mm, WW 1.2-1.5 mm, WC 2.6-3.3 mm (Table 2).

Colour. Living worms not seen. Alcohol preserved worms with light brown background, a prominent dark brown longitudinal mid-dorsal band along all body (figs. 1A, 1C, 2), and dark brown

Table 2. Variation in elytra distribution pattern and size in specimens of *Parahololepidella greeffi* associated to *Tanacetipathes* cf. *spinescens*. Asymmetrical and variable elytral positions are marked in italics. Width: WW/WC; R: right side; L: left side.

	Length (mm)	**Width (mm)**	**Chaetiger numb.**	**Elytra numb.**	**Distribution of elytra**
MNCN 16.01/ 13708 af	44	1.45/3.25	110	52	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 33 34 *38 40 42 44 46 48 50 52 54 56 60 62 64 66 68 7 2 74 76 78 80 82 84 86 88 90 92 94 96 98 100 102 104 106 109* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 33 34 *39 39 41 43 45 47 49 51 53 55 56 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96 98 100 102 104 106 109*
MNCN 16.01/ 13707	42		120	51/49	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 *33 35 39 42 44 46 48 54 56 58 60 62 64 66 68 70 72 74 76 84 87 90 92 95 97 99 101 102 103 105 107 108 110 112 116 119* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 *34 36 38 39 42 44 46 54 56 58 60 62 64 66 68 70 72 74 76 84 87 90 95 98 99 101 102 103 105 108 110 112 116 119*
MNCN 16.01/ 13705 af + pf	42	1.5/3.1	108	50/49	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 33 35 37 39 *41 43 45 47 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 81 83 85 87 89 91 93 95 102 104 106* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 33 35 37 39 *42 44 45 47 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 81 84 86 88 90 92 94 102 104 106*
MNCN 16.01/ 13704 af + pf	27	1.2/2.6	90	40	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 34 36 38 40 42 47 48 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 *83 86* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 34 36 38 40 42 47 48 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 *86 88*
NNMN 24481 af+ pf	35		99	43/45	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 *32 36 38 40 42 44 46 48 50 52 54 56 59 60 64 66 68 70 72 73 76 78 82 84 86 91 92 96 97* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 *33 34 35 37 39 41 43 45 47 49 51 53 55 57 59 60 63 65 67 69 71 72 75 77 81 83 85 89 92 96 97*
NNMN 24644	26		75	35/34	R 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 34 36 38 40 42 44 *46 48 50 52 54 56 58 60 62 64 66 68 70 72* L 2 4 5 7 9 11 13 15 17 19 21 23 26 29 32 34 36 38 40 42 44 *47 49 51 53 55 57 59 61 63 65 67 69 71*

pigmentation on cirrophores and, sometimes, on bases of cirri. Some specimens may also show a longitudinal dark brown band on ventral side, narrow in anterior segments, occupying nearly all body width from mid-body to posterior end (fig. 1B, 1D).

Remarks. Our specimens agree well with Pettibone's (1969) description. However, the syntype deposited at the ZMH was in a very poor state of preservation, being almost dehydrated (fig. 6A), to the extent that the the chaetae were damaged (fig. 6B). The material from the museum included a few dissected parapodia in an additional jar, which appeared to be in better conditions (fig. 6D-F). The only differences with the parapodia of the newly collected material were that the neurochaetae seemed to be slightly thicker in the syntype, two of them appearing slightly bidentate (black arrow, fig. 6E). Taking into account the conditions of this material, however, we cannot dismiss the possibility that these two traits could have been caused by the dehydrating process suffered by the syntype.

Some of the features commonly used to discriminate species and, even, genera among polynoids are highly variable within the newly collected material. For instance, specimen MNCN 16.01/137094 lacks cephalic peaks, while they are present in specimen NNMN 24644. Also, the elytra distribution becomes asymmetrical (within a given worm) and irregular (between worms) from chaetiger 32 or 33 to the end of the body (Table 2). A similar variability was also described for another long bodied species, *Medioantenna variopinta* (Di Camillo et al., 2011). The shape of elytra may also vary. They are relatively large, covering prostomium and mid-dorsum up to chaetigers 15–23, becoming then very small and leaving the dorsum uncovered. However, several specimens also show some small anterior elytra leaving the dorsum uncovered, we suggest this being caused by the presence of regenerating (small) elytra and/or parapodia. The restricted distribution of these damaged elytra and/or parapodia, lead us to attribute its presence to intra-specific aggressive

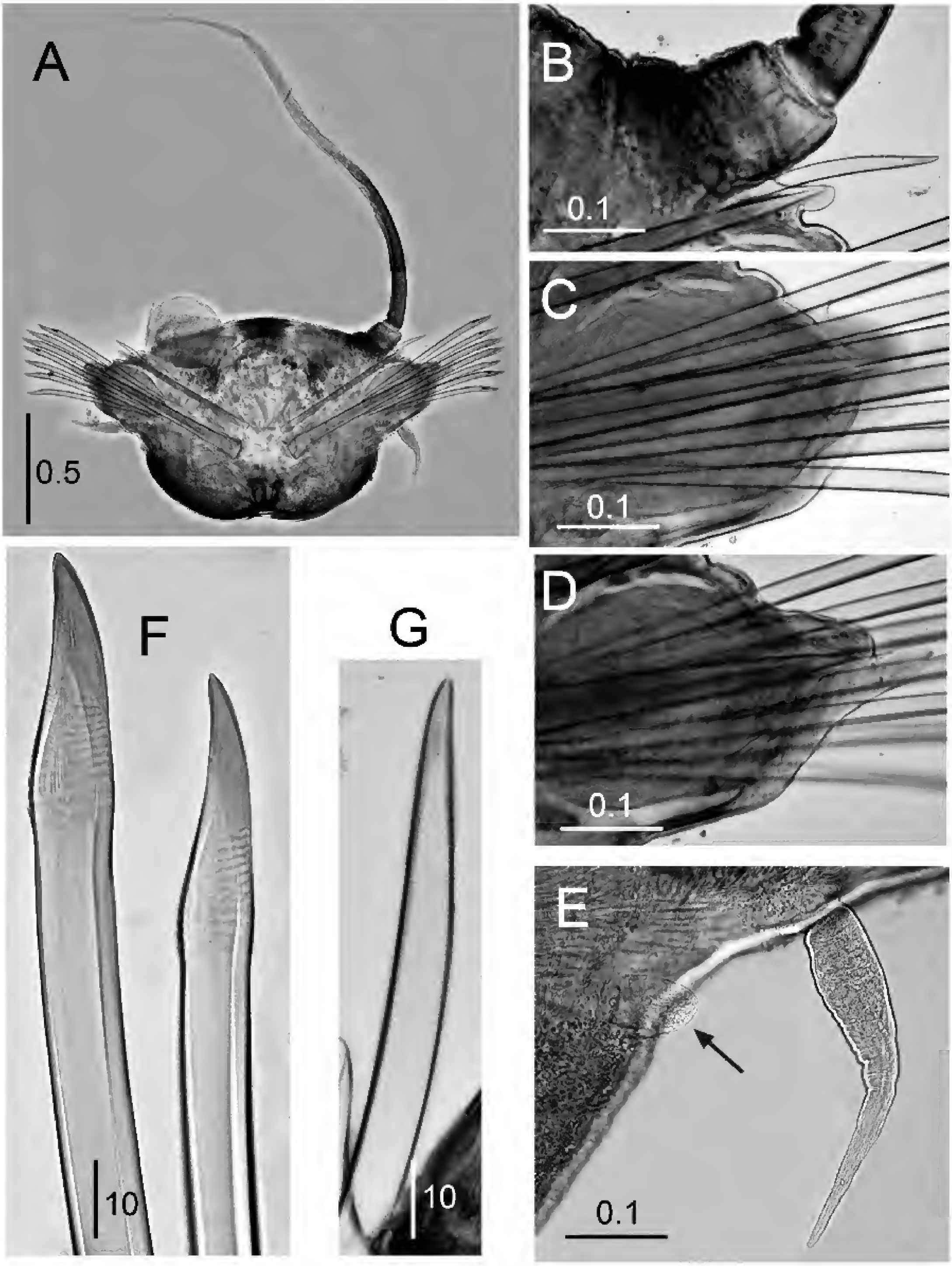

Figure 4.- *Paraholepidella greeffi*. MNCN 16.01/13708. Mid-body segment. A. Whole view of a transversal section, showing elytra and dorsal cirri on the same segment. B. Notopodium. C. Neuropodial acicular lobe. D. Neuropodial post-acicular lobe. E. Ventral cirri and nephridial papilla (black arrow). F. Neuropodial chaetae. G. Notopodial chaetae. Scale bars are cm (A) and mm (B-G).

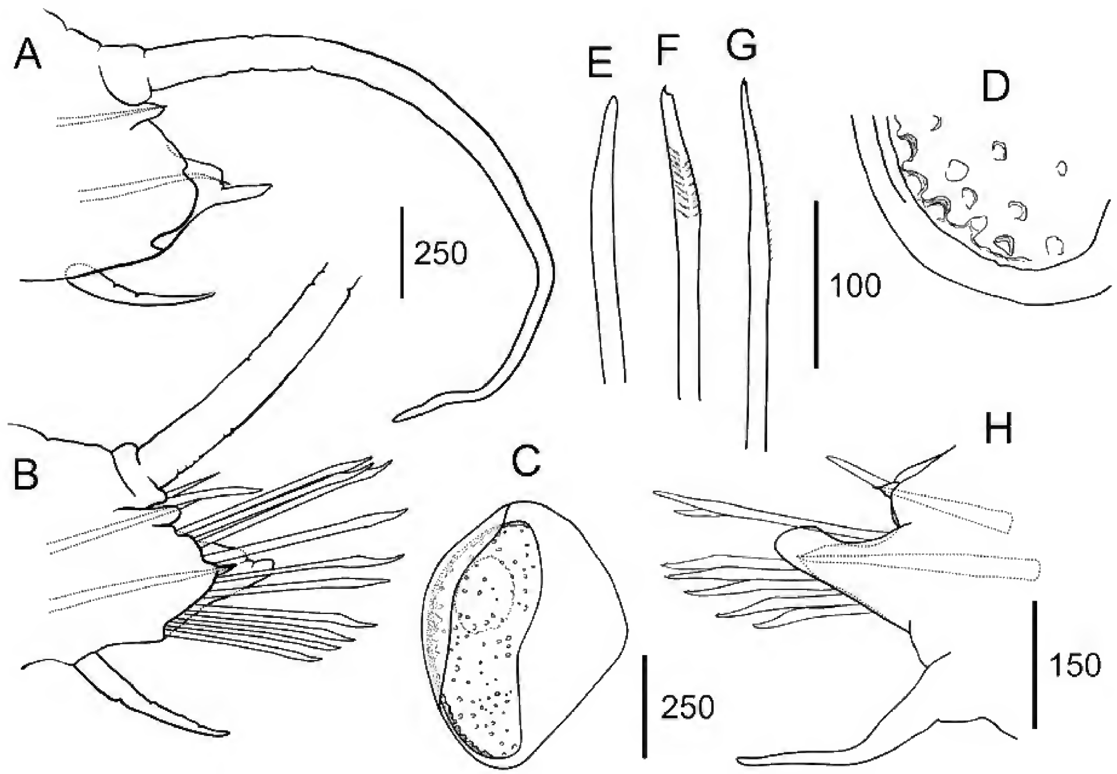

Figure 5.- *Parahololepidella greeffi*. MNCN 16.01/13708. Right cirrigerous parapodium. A. From chaetiger 44, posterior view, chaetae omitted. B. From chaetiger 12, posterior view, with chaetae. *Gorgoniapolynoe caeciliae*. MNCN 16.01/14337. C. Left elytron of first pair; left margin folded on dorsal side. D. Detail of margin of same elytron. E. Notochaeta. F. Ventral-most neurochaeta. G. Dorsal-most neurochaeta. H. 35th parapodium. Scale bars are μm.

behaviour that seems to characterize different species of symbiotic polychaetes, particularly polynoids (e.g. Britayev et al., 2007).

Elytrae also seem the reason why Rullier (1964) described a small (i.e. juvenile) specimen of *P. greeffi* as a new species, *Hololepidella fagei* Rullier, 1964. Being small, this specimen showed all elytra large, covering mid-dorsum, like those from anterior-most segments in larger worms. This species was synonymized with *P. greeffi* by Pettibone (1969) while describing *Parahololepidella* as a new genus.

Ecology. Parahololepidella greeffi was found at 0–30 m deep, living in association with colonies of the antipatharian *Tanacetipathes* cf. *spinescens*, while it was previously recorded as free living (Augener, 1918; Pettibone, 1969; Rullier, 1964). In fact, Rullier (1964) reported specimens of *H. greeffi* occupying white mucous tubes, incrusted with sand grains and fragments of shells. According to Pettibone (1969), these tubes were perhaps formed by some commensal host. However, the supposed presence of tubes was not observed in our material, as the worms were always directly attached to the surface of the host antipatharian, without any trace of tubes. They were crawling on the main stems of the plumose branches of the coral (fig. 7), having very similar, cryptic colour (when preserved). All six colonies examined harboured polychaetes, two of them with several individuals on each colony (up to 6 in a 15x10 cm branch, MNCN 16.01/13707).

As previously reported for all known symbiotic polychaetes (Martin & Britayev, 1998), the finding of *P. greeffi* as symbiont reinforces the high diversity of the representatives of the family Polynoidae living in association with antipatharian hosts: of the 12 known species, eight are polynoids, three are species of *Eunice*, and the remaining one is a syllid (Table 3).

Distribution. Tropical and Equatorial East Atlantic, Cabo Verde and São Tomé Archipelagos.

Genus ***Gorgoniapolynoe*** Pettibone, 1991

Type species. *Gorgoniapolynoe bayeri* Pettibone, 1991

Diagnosis. Body dorso-ventrally flattened, with up to about 60 segments; elytra leaving mid-dorsum uncovered, except in anterior-most segments. 15 pairs of elytra on chaetigers 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, and 32. First 1-2 pairs of elytra modified, with translucent, chitinous central area. Prostomium wider than long, with rounded lobes and three

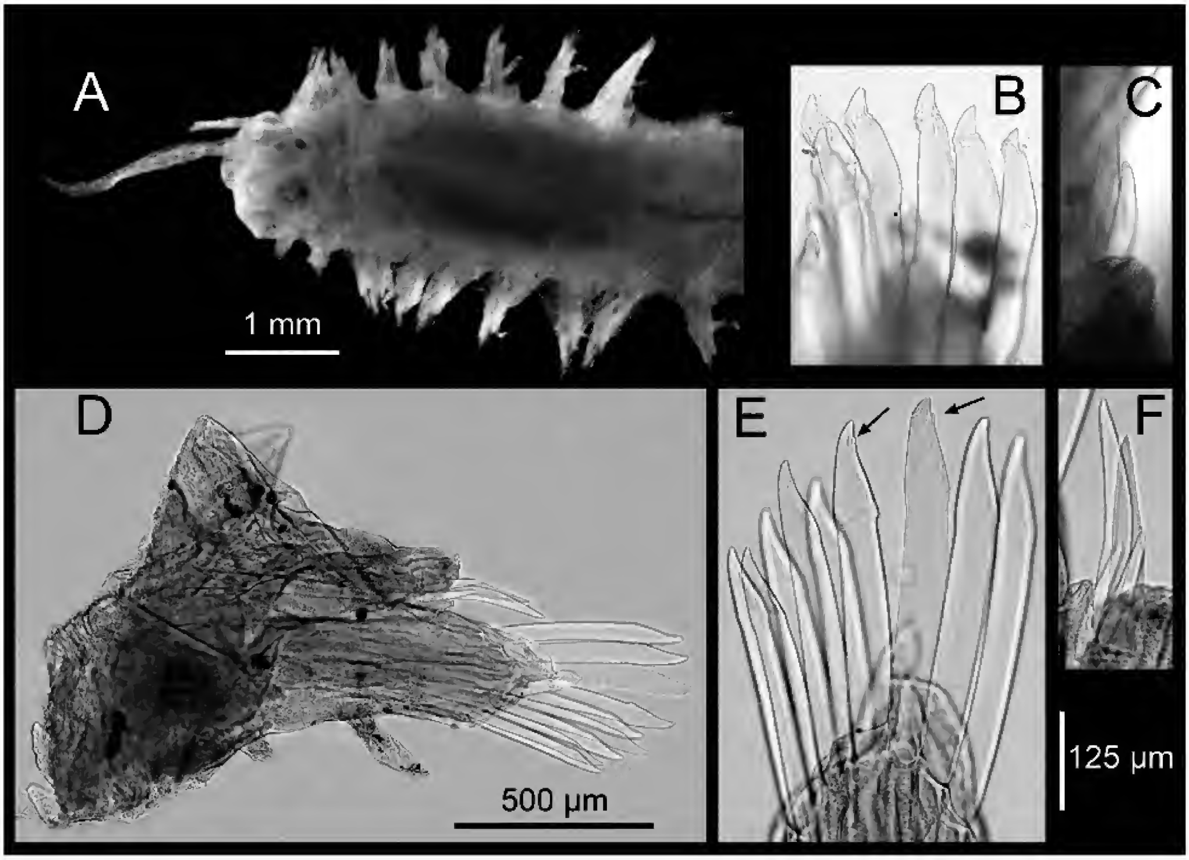

Figure 6.- *Paraholoepidella greeffi*. Syntype ZMH 5692. A. Anterior end, dorsal view. B. Neurochaetae from anterior region, showing damaged tips. C. Notochaetae. D. Dissected parapodia from mid-body. E. Neurochaetae of the same (black arrow pointing at the apparently bidentate chaetae). F. Notochaetae of the same. B, C, E, F: scale bar 125 μm.

antennae; cephalic peaks absent or present; lateral antennae latero-ventral to median antenna. Two pairs of eyes. Parapodia with elongate acicular lobes, with noto- and neuroacicula penetrating epidermis; tip of neuropodia extending to supra-acicular process. Notochaetae few (0–7), stout, with blunt tip; neurochaetae few, but more numerous (7-15), of same width as notochaetae, usually bidentate. Prominent glandular area on bases of ventral cirri starting from chaetigers 11-18.

Remarks. This diagnosis agrees in general with that of Pettibone (1991a) and Barnich et al. (2013). The former paper included nine species in *Gorgoniapolynoe*, among which seven fit well with the generic diagnosis thus forming a compact species group. However, *Gorgoniapolynoe corralophila* (Day, 1960) and *Gorgoniapolynoe pelagica* Pettibone, 1991a differ in several features. Both species have more numerous noto- and neurochaetae; *G. corralophila* has three pairs of modified elytra and notochaetae with widely spaced rows of spines and long bare tips. The single known specimen of *G. pelagica* is small, has twelve pairs of elytra and notochaetae of two kinds: long, stouter than neurochaetae, and short, of the same width as neurochaetae. This suggests that it could be a juvenile of another species. Accordingly, we propose that *G. corralophila* should be referred to a different genus and that *G. pelagica* could be a juvenile and thus the species should be considered as *nomen dubium*.

Gorgoniapolynoe caeciliae (Fauvel, 1913)

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(Figs. 5C-H, 8-10)

Polynoe caeciliae: Fauvel (1913), 24, fig. 7A–D; Fauvel (1914), 69, pl. 4, figs. 1-6, 18–19; Hartmann-Schröder, 1985: 31-33, figs. 1-5 (in part; not specimens from Indian Ocean, not figs. 6-11).

Gorgoniapolynoe caeciliae: Pettibone (1991a), 704, figs. 12-14.

Material examined. Galicia Bank, NW Iberian Peninsula. Host *Candidella imbricata*. MNCN 16.01/14337: 3 specimens from different colonies, Sta. DR10-14/08/2010, INDEMARES 2010 expedition, 1482 m depth, 42°27.672'N 011°59.233'W. MNCN 16.01/14338: 1 specimen from one colony, Sta. DR16- 24/08/2010, INDEMARES 2010

Table 3. List of known polychaete species associated with antipatharian hosts. 1) Hartmann-Schröder & Zibrowius (1998); 2) Molodtsova & Budaeva (2007); 3) Pettibone (1991b); 4) Wagner et al. (2012); 5) Pettibone et al. (1970); 6) Hanley & Burke (1991); 7) Barnich et al. (2013); 8) this paper; 9) Glasby (1994); 10) Glasby & Krell (2009).

Family	Species	Host	References
Eunicidae	*Eunice antipathum* (Pourtalès, 1867)	*Distichopathes filix* (Pourtalès, 1867)	1,2
		Elatopathes abietina (Pourtalès, 1874)	1,2
Eunicidae	*Eunice kristiani* Hartmann-Schröder & Zibrowius, 1998	cf. *Antipathes cylindrica* Brook, 1889	1,2
Eunicidae	*Eunice marianae* Hartmann-Schröder & Zibrowius, 1998	cf. *Antipathes cylindrica* Brook, 1889	1,2
Polynoidae	*Antipathypolyeunoa nuttingi* Pettibone, 1991b	*Tanacetipathes tanacetum* (Pourtalès, 1880)	3,4
Polynoidae	*Bayerpolynoe floridensis* Pettibone, 1991b	*Stylopathes columnaris* (Duchassaing, 1870)	3,4
Polynoidae	*Benhamipolynoe antipathicola* (Benham, 1927)	*Stylopathes tenuispina* Silberfeld, 1909	5
		Stylopathes columnaris (Duchassaing, 1870)	4,5
Polynoidae	*Brychionoe karenae* Hanley & Burke, 1991	*Leiopathes* sp.	6
Polynoidae	*Eunoe purpurea* Treadwell, 1936	*Bathypathes* cf. *alternata* Brook, 1889	7
Polynoidae	*Neohololepidella antipathicola* Hartmann-Schröder & Zibrowius, 1998	*Elatopathes abietina* (Pourtalès, 1874)	1,2
		Distichopathes filix (Pourtalès, 1867)	1,2
Polynoidae	*Parahololepidella greeffi* (Augener, 1918)	*Tanacetipathes* cf. *spinescens* (Gray, 1857)	8
Polynoidae	*Tottonpolynoe symantipatharia* Pettibone, 1991b	*Parantipathes* sp.	3
Syllidae	*Bollandiella antipathicola* (Glasby, 1994)	*Antipathes* sp.	2,9,10

expedition, 1423 m depth, 42°28.838'N 011°55.873'W.

Avilés Canyon System, Bay of Biscay, N Iberian Peninsula. Host *Corallium niobe*. MNCN 16.01/14341: 1 specimen, Sta. DR16-05/08/2010: 1 specimen from one colony fragment and several colony fragments without polychaetes but showing modifications of the axis front resulting from the interaction with the polychaetes, INDEMARES 2010 expedition, 928 m depth, 44°01.509'N 005°42.898'W.

Additional material: Voucher specimens deposited in the IEO laboratory, Gijón (Spain), and INSUB, Museo de Okendo, Donostia-San Sebastián. Galicia Bank, NW Iberian Peninsula. Host *Candidella imbricata*. Sta. DR10-14/08/2010: 32 specimens from seven colonies and fragments, INDEMARES 2010 expedition, 1482 m depth, 42°27.672'N 011°59.233'W. Sta. DR16-24/08/2010: 18 specimens from one colony and fragments, INDEMARES 2010 expedition, 1423 m depth, 42°28.838'N 011°55.873'W. Sta. DR04-22/07/2011: 1 specimen from one colony fragment, INDEMARES 2011 expedition, 1288 m depth, 42°58.419'N 12°02.982'W. Sta. DR12-05/08/2011: ca. 72 specimens from three colony fragments, INDEMARES 2011 expedition, 1585 m depth, 42°32.157'N 12°03.795'W. Host *Corallium* sp. Sta. DR08-13/08/2010: one dead colony with likely worm-induced galleries, without worms, INDEMARES 2010 expedition, 1196 m depth, 42°55.941'N 12°05.149'W.

Diagnosis. Prostomial lobes rounded, without cephalic peaks; first pair of elytrae modified with crescent shaped area on lateral side, transparent, chitinous, with scattered rounded microtubercles and elongate globular micropapillae (figs. 5C, 5D, 8A, 8B); remaining elytrae translucent almost circular with slightly folded borders (fig. 8C); dorsal cirri with scarce clavate papillae, mainly at basis (fig. 8D); parapodia as in generic diagnosis (figs. 5H, 8D), with big, digitate nephridial papillae (fig. 8E); 0–3 notochaetae, stout, with blunt tips (figs. 5E, 8F); 8–15 neurochaetae, as stout as notochaetae, bidentate (figs. 5F, 5G, 8G, 8H).

Measurements. 37-49 chaetigers, L 7.0-17.0 mm, WW 0.9-1.6 mm, WC 1.4-2.3 mm.

Remarks. The Iberian specimens agree well with the re-description of the species by Pettibone (1991a), except in the presence of clavate papillae on dorsal cirri, which were neither mentioned nor figured in the original description.

Ecology. *Gorgoniapolynoe caeciliae* lives in association with different species of octocorals belonging to the Acanthogorgiidae, Primnoidae and Coralliidae (Barnich et al., 2013; Bayer, 1964; Eckelbarger et al., 2005; Pettibone, 1991a). The polychaetes were observed in all sampling stations where the host *C. imbricata* (Primnoidae) was obtained, from 1288 m to 1585 m deep, living inside galleries formed by highly modified sclerites of the gorgonian (figs. 9A-9D), similar to those described by previous authors in the same host (see Cairns, 2004, on colonies from W Atlantic), but also on the acanthogorgiid gorgonian *Acanthogorgia armata* Verrill, 1878 and *A. aspera* Pourtalès, 1867, and on the primnoid gorgonian *Callogorgia* sp. (see Barnich et al., 2013; Britayev, 1981, and references herein).

Similar galleries (some with worms inside) were observed in other species of *Candidella*, such as *C. helmintophora*

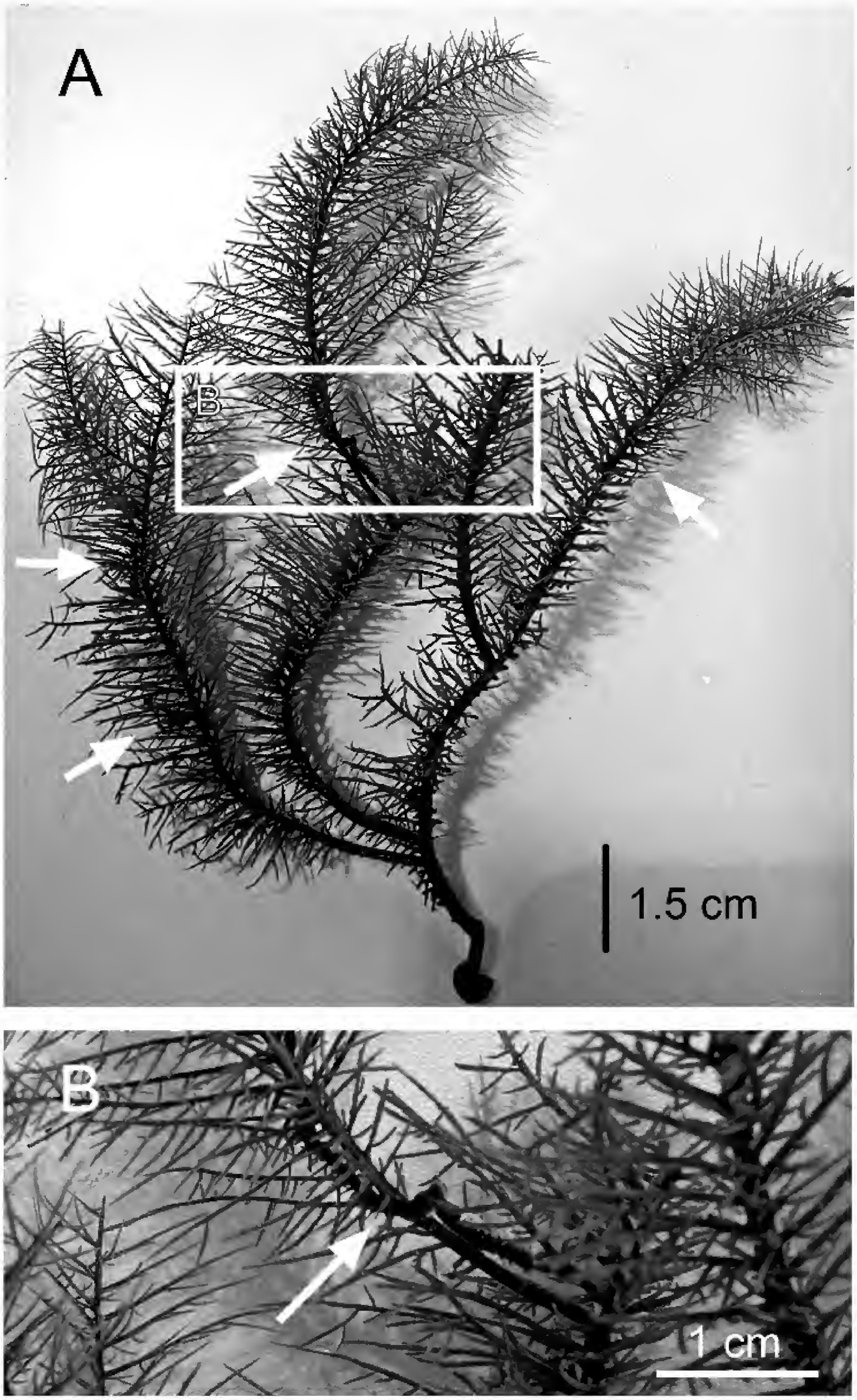

Figure 7.- *Tanacetipathes* cf. *spinescens*. MNCN16.01/13707. A.- Whole view of a host colony harbouring four specimens of *Parahololepidella greeffi*. B. Detail of a host curled on the main stem of the host black coral. White arrows point to the position of the symbionts.

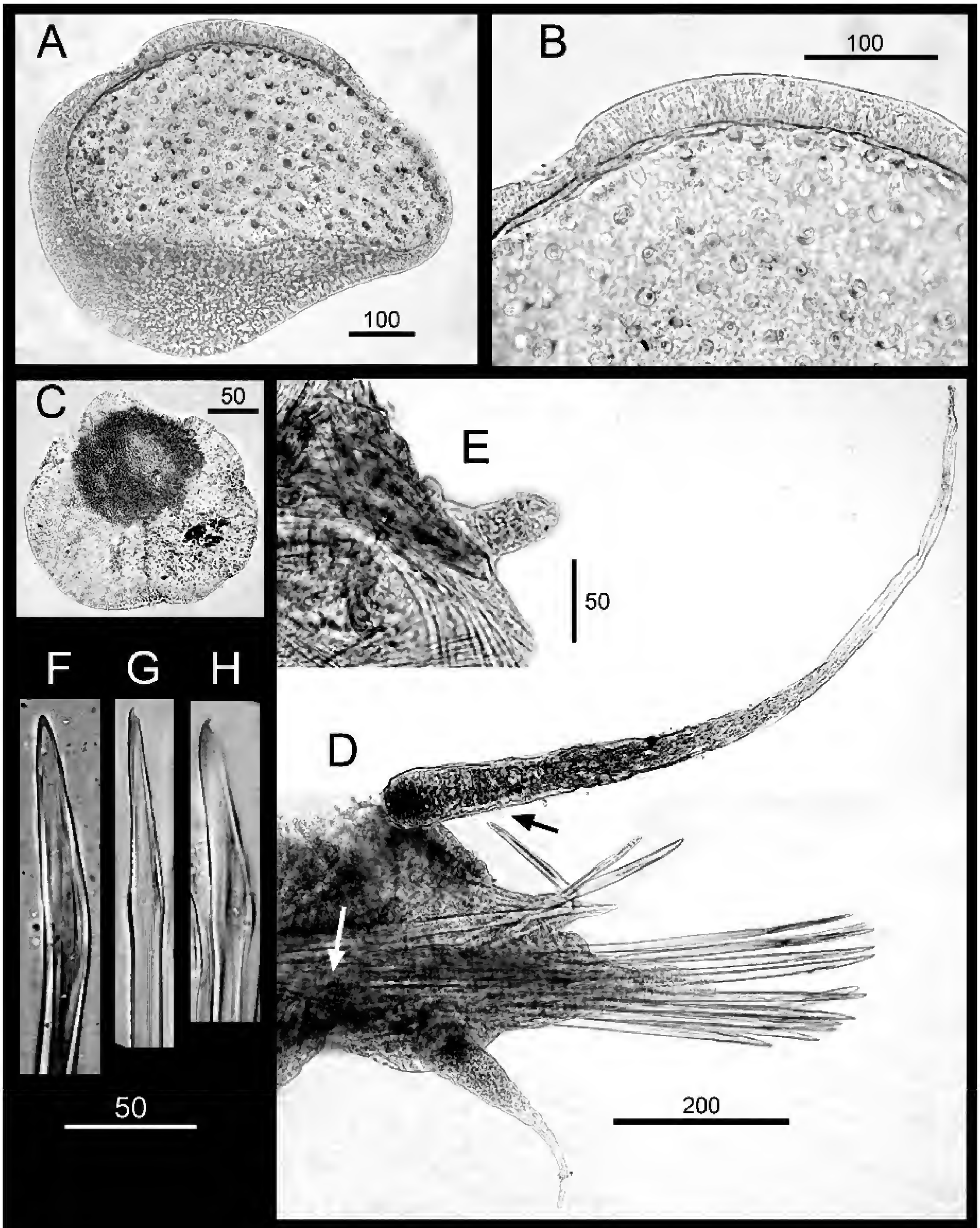

Figure 8.- *Gorgoniapolynoe caeciliae*. MNCN 16.01/14337. A. Left elytron from first pair. B. Detail of margin of same. C. Elytron from mid-anterior region. D. Parapodium from chaetiger 32, dorsal cirri broken (placed in approximate position); black arrow pointing on the small scattered papillae on cirri; white arrow pointing on the approximate position of nephridial papilla. E. Nephridial papilla. F. Notochaetae. G. Neurochaetae from dorsal-most bundle. H. Neurochaetae from ventral-most bundle. Scale bars are μm.

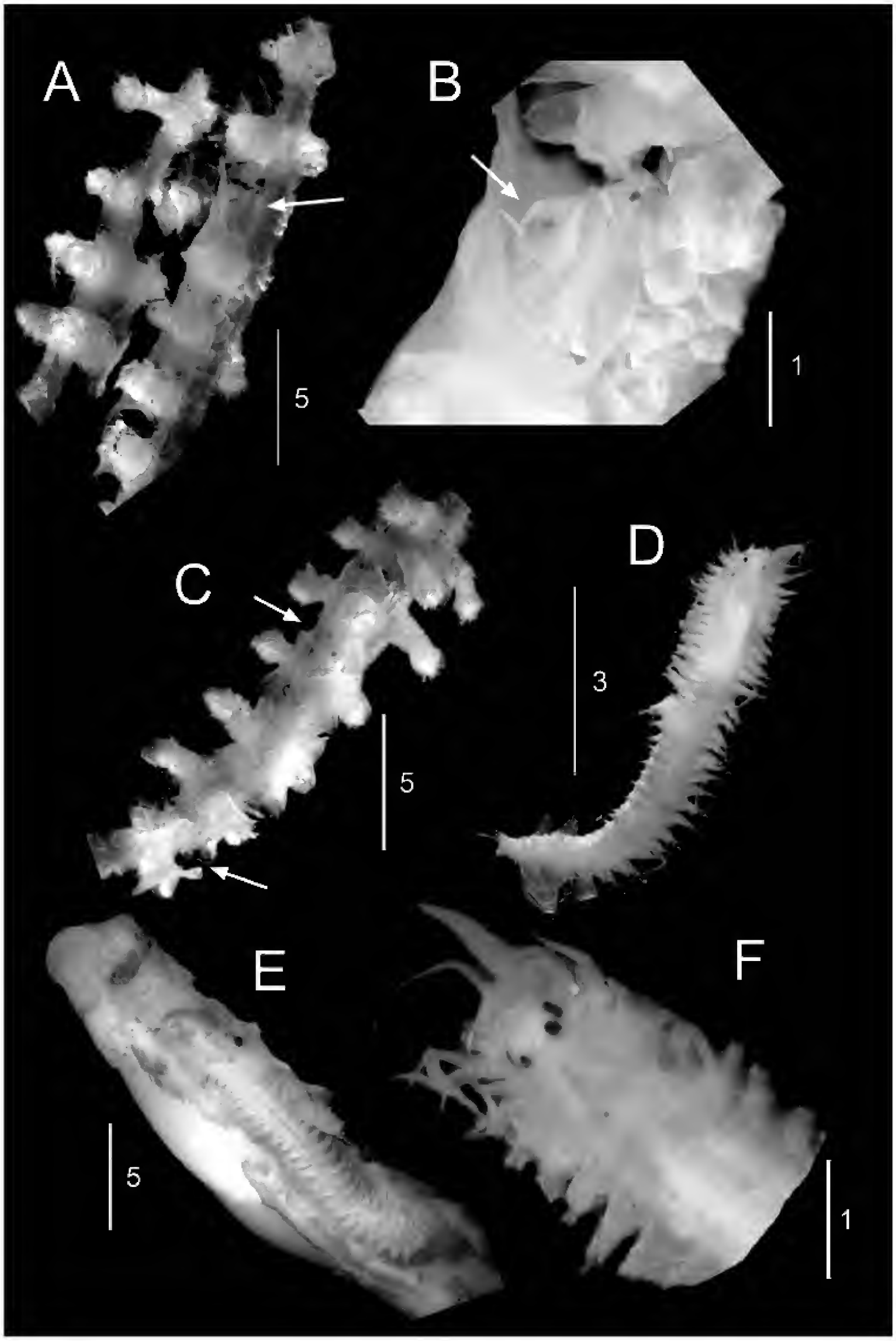

Figure 9.- *Gorgoniapolynoe caeciliae*. MNCN 16.01/14337. A. Two fragments of *Candidella imbricata*, one of them with the symbiont inside a gallery formed by expanded esclerites (arrow pointing on worm's head). B. Detail of the anterior end of the worm (arrow pointing on worm's head showing eyes through the first pair of elytra). C. Fragment of *Candidella imbricata* with the symbiont inside a gallery formed by expanded esclerites (arrows pointing on worm's head and pygidium). D. Same worm as in C, extracted from the gallery. MNCN 16.01/14341. E. Fragment of *Corallium niobe*, with a worm inside a gallery in the axis of a branch. F. Anterior end of the same worm as in E, extracted from the gallery. Scale bars are mm.

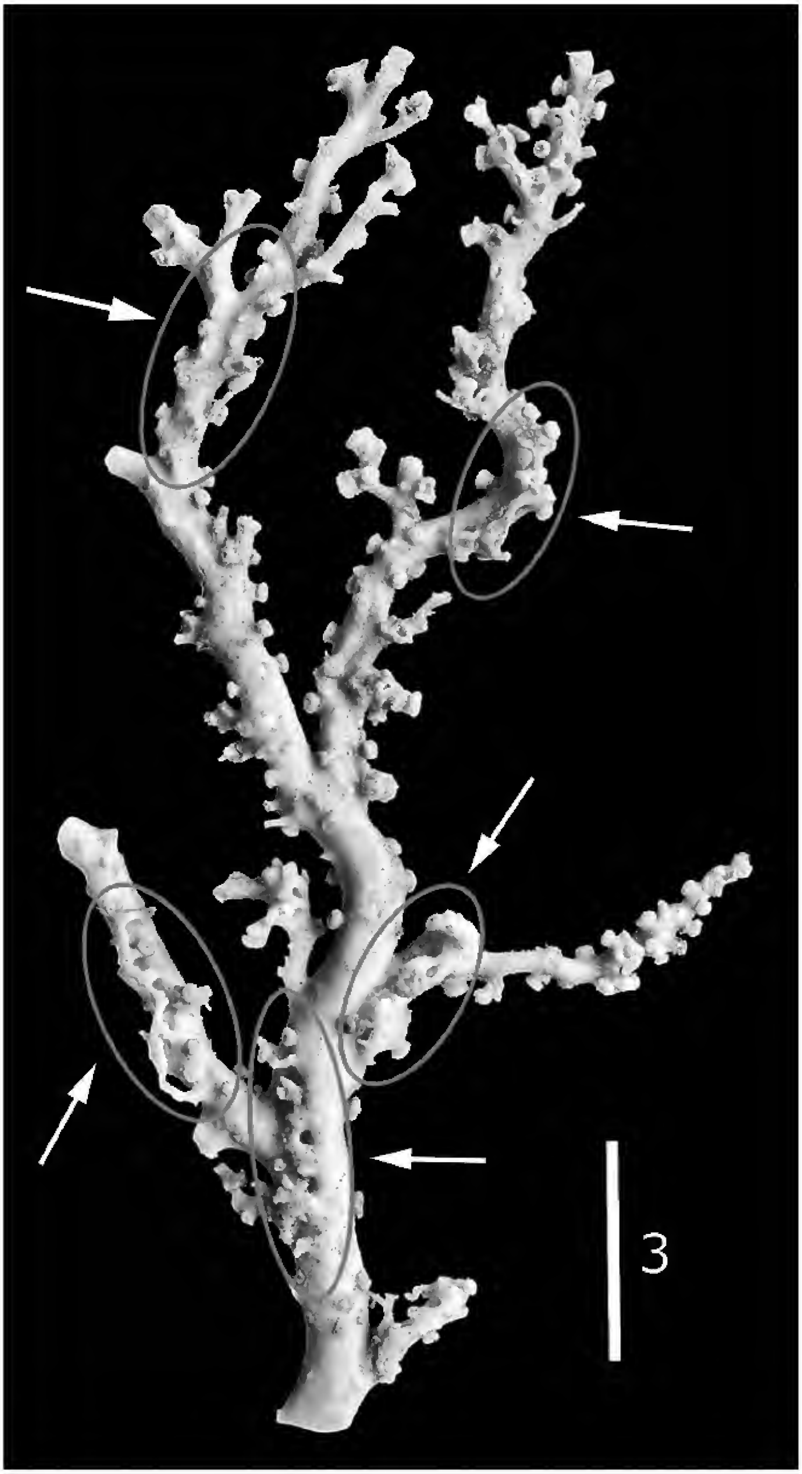

Figure 10.- Dead colony of *Corallium* sp. showing traces of galleries (pointed by white arrows and outlined by red tracing) likely originated for the association with *Gorgoniapolynoe caeciliae*. Scale bar is cm.

(Nutting, 1908) from Hawaii (Cairns, 2009; Nutting, 1908). Other Hawaiian gorgonians, belonging to the genus *Narella* (Primnoidae), such as *N. alata* Cairns & Bayer, 2008, *N. macrocalyx* Cairns & Bayer, 2008 and *N. vermifera* Cairns & Bayer, 2008 (Cairns & Bayer, 2008), showed similar galleries with worms. However, the polychaetes in these four host species were not identified. Thus, it is not possible to assess whether they belong to the same polynoid species or to a similar one. For instance, *Gorgoniapolynoe galapagensis* Pettibone, 1991a was described in association to *Narella ambigua* (Studer, 1894) from Galapagos Islands (Eastern Central Pacific Ocean) and *Gorgoniapolynoe bayeri* Pettibone, 1991a, associated with *Narella clavata* (Versluys, 1906), occurred in Philippine Islands (North Pacific Ocean).

Gorgoniapolynoe caeciliae was also reported in association with five species of *Corallium* (Coralliidae), *C. bayeri* Simpson & Watling, 2011, *C. johnsoni* Gray, 1860, *C. niobe* Bayer, 1964, *C. secundum* Dana, 1846 and *C. tricolor* (Johnson, 1898) (Bayer, 1964; Fauvel, 1913; Hartmann-Schröder, 1985; Simpson & Watling, 2011; Stock, 1986). It must be pointed out that Stock (1986) reported *C. profundum* Dana, 1846 as a host for the polychaete, but this species does not exist and most likely was a misspelling for *C. secundum.* When associated with *Corallium*, including our sample of *C. niobe* (figs. 9E, 9F), the worms induce malformations in the host branches, which form entirely covered galleries that contain a single worm inside (see Barnich et al., 2013, and references herein). Similar galleries were also depicted by Bayer (1956) on *C. secundum* and Bayer (1964) on *C. niobe*, but the worms were not identified. The dead colony of *Corallium* found in Galicia Bank completely lacked the original soft tissues (those observed in the picture correspond to secondary colonization of the coral skeleton by a zoantharian), this preventing the identification to species level. However, the skeleton also showed traces of several galleries (fig. 10), which agree with those found on the living colonies of *C. niobe* harbouring the polychaete at the Avilés Canyon System.

In all cases, all the galleries were not excavated on the coral skeleton but appeared to be produced by the coral tissues and skeleton overgrowing the original soft tube produced by the worm (which may still be observed laying between the coral tissues and the worms themselves), in a similar way to the modifications induced by *Eunice norvegica* (Linnaeus, 1767) on its host scleractinian coral *Lophelia pertusa* (Linnaeus, 1758) (Mueller et al., 2013). This suggests that *G. caeciliae* may play an equivalent, functional role to that of *E. norvegica* in structuring the assemblages of its coral hosts.

Distribution. Widely distributed in the NW and NE Atlantic, from 400-1500 m depth according to Barnich et al. (2013). The present report includes a slightly deeper depth range (down to 1585 m) and is the first mention of the association between *G. caeciliae* and *C. imbricata* for Spanish waters. The presence of the polychaete in different locations from N and NW Iberian waters was previously reported by Fauvel (1913), Hartmann-Schröder (1985) and Pettibone (1991a) in association with *Corallium* species (i.e. *C. niobe* and *C. johnsoni*).

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Morphological anomalies in polychaetes: *Perinereis* species (Polychaeta: Annelida) examples from the Brazilian coast

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Abstract Coutinho, M.C.L. and Santos, C.S.G. 2014. Morphological anomalies in polychaetes: *Perinereis* species (Polychaeta: Annelida) examples from the Brazilian coast. *Memoirs of Museum Victoria* 71: 45–51.

The examination of a large number of specimens in the context of taxonomic and ecological studies may lead to the discovery of morphological anomalies. The aim of this study was to describe the morphological anomalies observed in some individuals of *Perinereis anderssoni* and *Perinereis ponteni* collected in various regions of the Brazilian coast. A total of 290 specimens were analysed from along the northern and southern Brazilian coast, and 21 of these presented morphological anomalies, such as variations in the number of tentacular cirri and eyes, completely or basally fused antennae, chaetigers with three parapodia, and others. *Perinereis anderssoni* presented the highest number of anomalous individuals, and the most frequent morphological anomaly was the presence of a single antenna and nine tentacular cirri. Anomalous individuals of *P. ponteni* with seven tentacular cirri were also commonly collected. Ilha do Mel (PR) was the area with the highest percentage of individuals with anomalies (12.96%), followed by Martim de Sá (SP) (10.31%), São Francisco do Conde (BA) (8.33%), Tambaba (PB) (5.55%) and Itaipu (RJ) (1.92%). Most of the sampling locations have a history of contamination by a diverse array of pollutants. We provide background information for the morphological changes observed in two species that occur along the Brazilian coast, but additional studies are needed to confirm the real cause of these anomalies and their effect on the population structure of these ecologically important species.

Keywords morphology, abnormalities, Nereididae, *Perinereis*, *Ceratonereis*, *Unanereis*

Introduction

The genus *Perinereis* is commonly found in shallow-water environments is composed of approximately 60 described species and is considered polyphyletic (Bakken and Wilson, 2005). The species in this genus are characterised by the presence of a proboscis with conical paragnaths on the maxillary and oral rings, and conical and additional bar-shaped paragnaths on the oral ring, four pairs of tentacular cirri with distinct cirrophores, one pair of biarticulated palps, a pair of frontal antennae, two pairs of eyes, notopodia with homogomph spinigers throughout, neuropodia with homogomph spinigers, heterogomph spinigers and heterogomph falcigers, notopodial ligule present and prechaetal notopodial lobe and postchaetal neuropodial lobe present or absent (De Léon-González and Solís-Weiss, 1998; Bakken and Wilson, 2005).

The examination of a large number of specimens in the context of taxonomic and ecological studies may lead to the discovery of morphological anomalies (Mohammad, 1981). These anomalies may occur within and between populations and can be the result of a range of processes (e.g. genetic, ecophenotypic or ontogenetic) or due to other factors, such as injury. Genetic processes are related to the presence of different genotypes in the same population or different populations of the same species, and are associated with phenotypic (i.e. relating to the external shape, physiological or behavioural character) variations of adaptive value to individuals. Morphological changes with adaptive value have been found in several species of polychaetes, particularly in the Nereididae (Geracitano et al., 2004a). Ecophenotypic factors refer to morphological changes resulting from environmental changes, such as contamination or changes in the concentration of an abiotic factor, and they can also generate genetic alterations (Backmann et al., 1995; Geracitano et al., 2002, 2004b; Bocchetti et al., 2004; Ferreira-Cravo et al., 2009; Mouneyrac et al., 2010; Ahrens et al., 2013). Ontogenetic factors are associated with the changes undergone by organisms during their development (Qian, 1999; Kubal et al., 2012). In the same way, injuries caused by predators may alter the bodies of individuals and

result in the reduction or absence of cirri and parapodial structures.

The aim of this study was to describe the morphological anomalies observed in individuals of *Perinereis anderssoni* Kinberg, 1866 and *Perinereis ponteni* Kinberg, 1866, collected from different regions along the Brazilian coast. It is beyond the scope of this study to determine the causes of these anomalies.

Materials and methods

A total of 119 atokous individuals of *P. ponteni* (7.00–77.50 mm) and 171 of *P. anderssoni* (4.50–58.12 mm) from different states along the northern and southern Brazilian coast were analysed. Specimens of *P. anderssoni* were collected from four populations in the following localities: Ilha do Mel, Paraná (PR) (July to August 2012); Itaipu, Rio de Janeiro (RJ) (April 2009 to April 2010); Tambaba, Paraíba (PB) (February 2009); Martim de Sá, São Paulo (SP) (March, April, August and September 2001). Specimens of *P. ponteni* were collected from four populations in the following localities: Ilha do Mel, Paraná (PR) (July 2012); Itaipu, Rio de Janeiro (RJ) (August 2009 to March 2012); São Francisco do Conde, Bahia (BA) (July 2011) and Martim de Sá, São Paulo (SP) (March and September 2001) (fig. 1).

All specimens were collected from rocky shores by scraping small areas covered by the bivalve *Brachidontes* sp. and the green alga *Ulva* sp. and mixed with coarse sediment grains. Specimens were anesthetized with menthol, fixed in 10% formalin (except for the populations of Itaipu, which were fixed in 4% formalin) and preserved in 70% ethanol. The specimens were examined with a stereomicroscope and photographed with a Sony CyberShot 13MP digital camera. Photographs were edited with PhotoScape v.3.6.2.

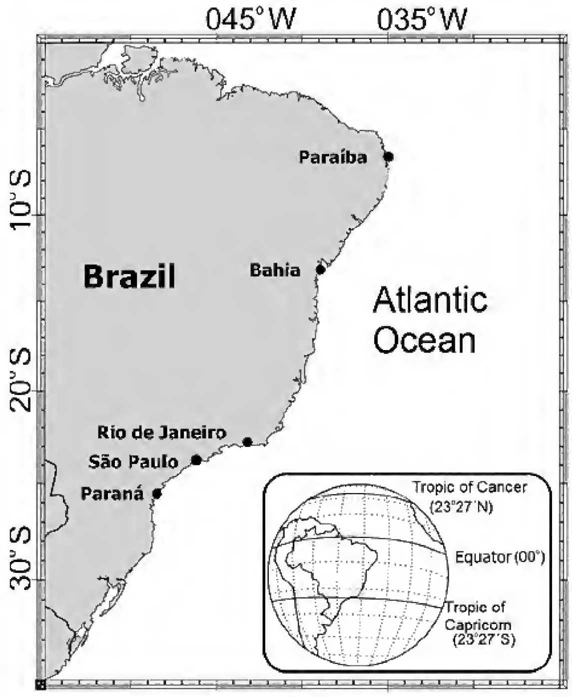

Figure 1. Location of sampling sites in states of Brazil.

Results

A total of five specimens of *P. ponteni* (9.8–20.6 mm long) and 16 specimens of *P. anderssoni* (6.0–41.9 mm long) from a number of populations presented morphological anomalies (figs 2 and 3). Based on descriptions of *P. anderssoni* and *P. ponteni* by Lana (1984), De Leon-González (1999) and Santos and Steiner (2006), the differences were considered anomalies and not intraspecific or interspecific variations as they did not follow a pattern of occurrence (table 1). It is also notable that the cirri and parapodial anomalies were never symmetrical, nor did they occur in the same parapodia or body region.

Of the two species, *P. anderssoni* had the most observed anomalies, and this may be partly explained by the greater number of individuals examined. Among the sampling localities, Ilha do Mel (PR) was the locality with the highest percentage of anomalous individuals (12.96%), followed by Martim de Sá (SP) (10.31%), São Francisco do Conde (BA) (8.33%), Tambaba (PB) (5.55%) and Itaipu (RJ) (1.92%) (fig. 4).

Table 1. Morphological characteristics and anomalies described in P. ponteni and P. anderssoni

Morphological characters/ Species	Normal characters	Anomalies	
		P. ponteni	*P. anderssoni*
Antennae	A pair of frontal antennae		Single antenna, two antennae completely fused, two antennae basally fused
Tentacular cirri (number)	Eight tentacular cirri	Seven or nine tentacular cirri	Six, seven or nine tentacular cirri
Number of parapodia	Chaetiger with two parapodia		Chaetiger with three parapodia
Number of eyes	Two pairs of eyes		Five eyes

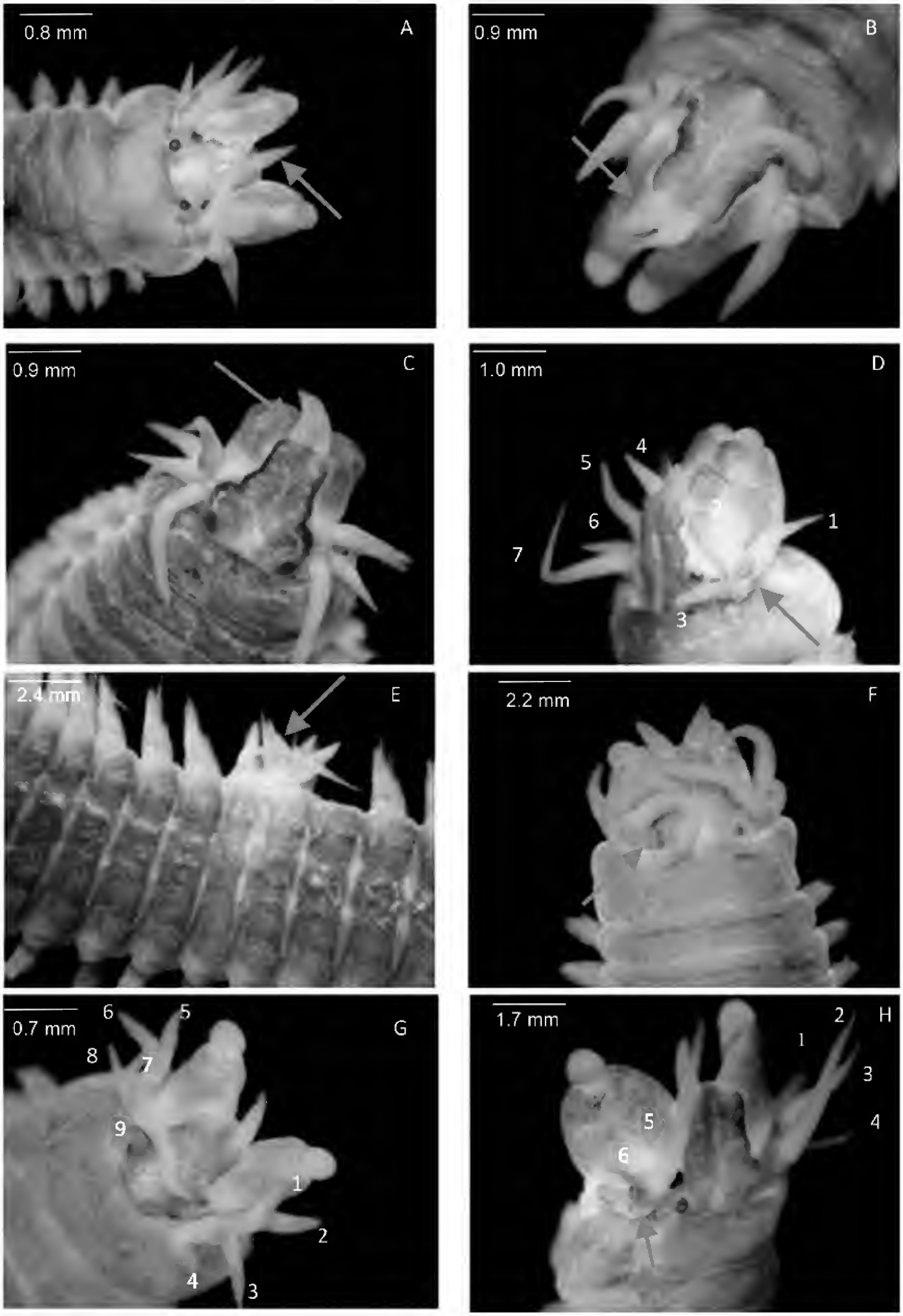

Figure 2. Morphological anomalies in *P. anderssoni*: A. single antenna; B. basally fused antennae; C. completely fused antennae; D. seven tentacular cirri; E. two parapodia on the same side of chaetiger; F. five eyes; G. nine tentacular cirri; H. six tentacular cirri.

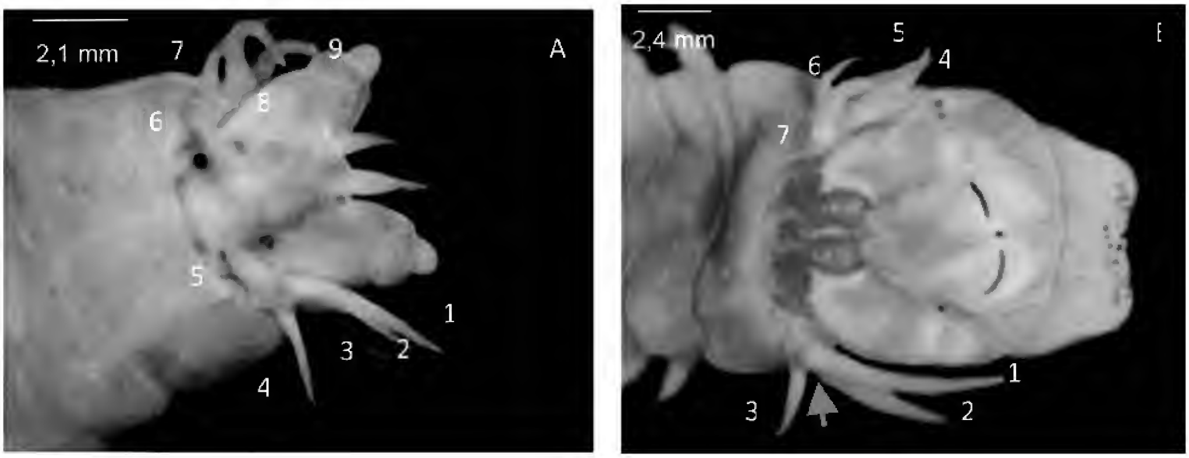

Figure 3. Morphological anomalies in *P. ponteni*: A. nine tentacular cirri; B. seven tentacular cirri.

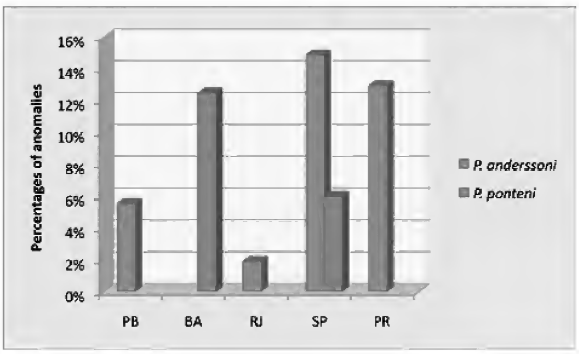

Figure 4. Percentage of morphological anomalies found in the species *P. anderssoni* and *P. ponteni.*

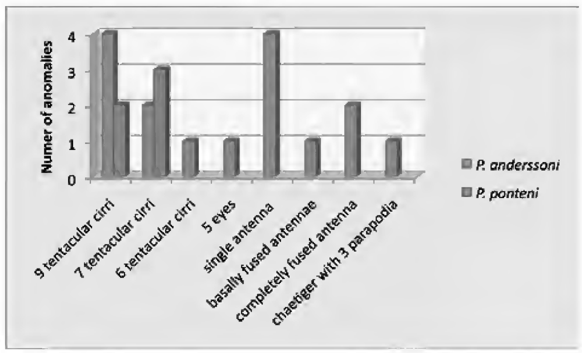

Figure 5. Number and type of morphological anomalies found in the species *P. anderssoni* and *P. ponteni.*

The most frequently observed morphological anomaly shared by both species was the presence of nine tentacular cirri. For *P. anderssoni*, the most frequent anomaly was the presence of a single antenna and nine tentacular cirri, and for *P. ponteni*, it was the presence of seven tentacular cirri (fig. 5). In some specimens, we found alterations in the number of paragnaths, but this was not considered anomalous.

Discussion

It is beyond the scope of this paper to determine the possible causes of the morphological anomalies that were observed. Most studies that report malformations or anomalies in polychaetes relate them to exposure to pollutants and its effects at many levels: individual, specific, population and community. In an earlier study, Reish et al. (1974) observed bifurcation in *Capitella capitata* larvae exposed to copper and zinc. Geracitano et al. (2004b) found morphological and histological anomalies (e.g. curling, protrusions, cuticle separation from the epidermis) in *Laeonereis acuta* that were caused by copper exposure. In addition, Méndez et al. (2009) described changes in colouration, swelling and rupture of the epidermis in *Eurythoe complanata* individuals exposed to mercury. Oliveira (2009) associated the anomalies observed in *Laeonereis* species (such as hypertrophy of the cirri and dorsal lobes, absence of dorsal ligules, and bifurcated cirri, lobes and ligules) with environments polluted by domestic and industrial sewage and harbour activities.

The sampling localities in this study show variation in their degree of ‘health’. The economy of Ilha do Mel, for example, is based on tourism, and the region suffers from the influence of Paranaguá Bay, where fishing, urban occupation, tourism and industry are all common activities, and the bay is home to the main South American grain shipping port (Martins et al., 2010; Gonzaga et al., 2013). Prior information about morphological changes in some marine organisms in the area is available in Valdez-Domingos et al. (2007), who recorded histopathological lesions in the gills of *Crassostrea rhizophorae* found in Paranaguá Bay. There was no direct relationship established by the authors between the lesions and

a specific contaminant because the study only evaluated the impact of a range of human activities. Among the species we studied, a single antenna was the anomaly most observed in polychaetes from this locality.

Fishing is the main economic activity in Itaipu followed by tourism. However, this locality is adjacent to Guanabara Bay, which is considered one of the most polluted environments of the Brazilian coast. It hosts large municipalities, several industries, shipyards, ports, naval bases, refineries and marine oil terminals (Marques-Júnior et al., 2009). There are already local records of histopathological alterations in a species of commercial fish (Cardoso et al., 2009) contaminated by mercury. The most common morphological anomaly found in polychaete specimens from Itaipu Beach was the presence of a single antenna.

There is one genus in the family, *Unanereis* Day, 1962, with two described species: *U. macgregori* Day, 1962 and *U. zgahli* Ben Amor, 1980, that presents one antenna. The first species description was based on an incomplete specimen, and the second description is poor. Apart from the number of antennae, all of the other *Unanereis* characteristics are similar to *Ceratonereis* species, including long tentacular and notopodial cirri. The second species description is also based on one specimen, and apart from noting the presence of one antenna, it is similar to *Composetia* Hartmann-Schröder, 1985, which was previously considered a subgenus of *Ceratonereis*. The author mentioned that the species is similar to *Ceratonereis costae* Grube, 1840 in all other features. Bakken and Wilson (2005) and Santos et al. (2005) nested both genera, *Unanereis* and *Ceratonereis*, in a polytomy. They did not make any decisions about nomenclature, and that is not our intention here, even though it deserves attention. We suggest, based on what we have observed for *Perinereis* species, *Pseudonereis* and *Laeonereis* (Santos and collaborators, pers. obs.), that until more *Unanereis* specimens are found, the presence of one antenna could, in fact, be an anomaly and not a synapomorphy of this taxon.

In São Francisco do Conde, oil-related activities are potential sources of pollution in the region (Veiga, 2003). Santos (2011) reported cases of cadmium and lead contamination in fish and shellfish in a number of localities in São Francisco do Conde. Specimens from this locality presented variations in the number of tentacular cirri as their most common anomaly.

Tourism is also the main economic activity in Tambaba and Martim de Sá (Projeto Orla). In Martim de Sá, fishing activities are also important (http://www.caraguatatuba.sp.gov.br). Specimens collected in this region presented variations in the number of tentacular cirri. We believe that genetic or ecophenotypic factors may be responsible for the larger numbers of tentacular cirri because this anomaly was found in both Martim de Sá, where there are no records of severe contamination, and in São Francisco do Conde, an affected site. Lower numbers of tentacular cirri can be explained by injury, such as from predation, but the same cannot be said when a larger number of cirri are observed.

Others anomalies found in this study, such as a variation in the number of eyes, may be due to genetic factors; this anomaly was found in Martim de Sá, which has not experienced high levels of contamination.

Historically, variation in the number of paragnaths is considered important and of taxonomic value as it is a diagnostic trait in some species. Small variations in quantity within or among populations are usually considered normal. According to Ben-Eliahu (1987), the number of paragnaths in proboscidial areas can be size-related. Breton et al. (2004) identified variations in the number of paragnaths in populations of *Nereis virens* from different sites and concluded that the differences were due to intraspecific variation. Garcia-Arberas and Rallo (2000) associated the change in the number of paragnaths in *Hediste diversicolor* with change in environmental conditions, such as sediment grain size. Maltagliati et al. (2006) also studied the paragnaths of *H. diversicolor* and suggested that paragnaths on different rings (oral and maxillary) may have different functions. This species has a variable diet, and one possible cause for variation is heritability of different patterns of paragnaths. However, previous morphometric analysis carried out by Coutinho (2013) (using the same individuals used here) and by Clímaco (2013), for *Allita succinea*, found no relationship between size or age and the number of paragnaths encountered. Silva (2014), in a phylogeographic study of *P. anderssoni* and *P. ponteni* along the Brazilian coast, suggested that *P. ponteni* is the same species found all along the coast and that *P. anderssoni* consists of two, latitudinally separated cryptic species: one species is found in the north-east, and another is distributed in the south and south-east of Brazil. Nevertheless, we found no congruence between the variability in the number of paragnaths and the distribution suggested by Silva (2014). Once, four paragnaths in area V were found in individuals from Itaipu (south-east) and Tambaba (north-east), and individuals from Ilha do Mel (south) presented either five or two paragnaths in area V. Therefore, the variation in the number of paragnaths observed in the specimens studied here is not considered anomalous but normal morphological variability.

It is reasonable to assume that ecophenotypic factors, such as pollution, could be generating the observed morphological changes in these species, because benthic organisms are more sensitive to environmental changes as a result of their limited mobility.

We dismissed ontogenetic factors and methods of fixation as possible causes of the anomalies found in this study. Peixoto (2013) described the reproductive biology and population structure of *P. anderssoni*, and none of these anomalies or morphological alterations was found in the larvae or small size-classes; they were found in adult specimens (Peixoto, pers. com.). For *P. ponteni*, ontogenetic factors may be possible, but this is improbable because this species is morphologically similar to *P. anderssoni*. Until now, no alterations of this magnitude have been linked to methods of fixation, so we also dismiss this factor.

Based on our results and the information discussed above, we suggest that more studies are needed to confirm the real causes of the morphological anomalies found in *P. anderssoni* and *P. ponteni*. Additionally, other localities, including pristine rocky shores, should be investigated along the Brazilian coast, as both species are widely distributed and common in shallow-water environments.

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Oogenesis in *Phragmatopoma* (Polychaeta: Sabellariidae): Evidence for morphological distinction among geographically remote populations

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Abstract

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The Southwest Atlantic Ocean sand-reef building polychaete, *Phragmatopoma lapidosa*, was recently synonymised with *Phragmatopoma caudata* based on morphological characters. This study uses histochemical and ultrastructural procedures to describe oogenesis in *Phragmatopoma caudata* from the Southwest (SW) Atlantic and make a comparison with previously published data for the Northwest Atlantic (NW) forms. In the South American worms, the exposed ovary consists of simple groups of oogonia attached to blood vessels, unlike the NW Atlantic worms in which only the proliferative and previtellogenesis phases of the oocytes are associated with blood vessels. In SW Atlantic worms, the oocytes float in the coelom during the vitellogenic phase. We discovered several heterogeneous features (e.g., cell extensions, amoeboid cells, ovary capsule, active uptake of material from blood vessels and egg envelope) that can be used to distinguish between North and South Hemisphere populations of *P. caudata*. In light of the observed divergence between worms from these separated populations, our findings support reproductive plasticity. The present study reveals biodiversity within sand-reef making sandcastle worms.

Keywords ultrastructure, histochemistry, reproductive biology, ovary,geographic plasticity, histology, benthic invertebrates, worm reefs.

Introduction

The type-locality of *Phragmatopoma lapidosa* Kinberg, 1866, is Rio de Janeiro, Brazil. The species was synonymised with *P. caudata* Krøyer in Mörch (1863) described from a population in the North Atlantic Ocean (Kirtley, 1994). The same author synonymised *P. moerchi* Kinberg, 1867, *P. digitata* Rioja, 1962 and *P. peruensis* Hartman, 1944 with *P. virgini* Kinberg, 1867 (Kirtley, 1994), demonstrating a different approach to species discrimination in the genus. Systematic studies among *Phragmatopoma* spp. have focused on characters of the anterior region of the body, especially the modified opercular paleae (Amaral, 1987, Hartman, 1944, Kinberg, 1867, Kirtley, 1994, Mörch, 1863). However, there is no concise comparative morphological study on these structures documenting clear variability among and within various *Phragmatopoma* species. Probably because of this, the taxonomy of *Phragmatopoma* spp. is incomplete and imprecise. Several species of sabellariids appear to have been described inadequately and redescription seems to be needed (Kirtley, 1994).

In the past, it was assumed that different strategies of reproductive biology were reliable for delineating species. Therefore, oogenesis and ovary structure in several polychaetes were reviewed, and a summary phylogeny was proposed (Eckelbarger, 1983, 1984, 2005, 2006). Light microscopy and ultrastructural studies of ovary morphology and development were carried out on several species from different polychaete families representing a wide-spectrum sampling of reproductive biology. Among the different species of *Capitella* Blainville, 1828, specific types of yolk precursors and metabolites were uptake by the oocyte during vitellogenesis suggesting variation in the egg envelope. Differentiation of the oocyte occurs

following separation from the follicle cells (Eckelbarger and Grassle, 1983). Studies of oogenesis (Eckelbarger, 1979) and larvae (Eckelbarger, 1976, Eckelbarger and Chia, 1978, McCarthy *et al.*, 2002) in *Phragmatopoma lapidosa* (syn. *P. caudata*) of the Northwest Atlantic Ocean described the regional pattern of reproduction and development. Eckelbarger (1976) observed for North American *Phragmatopoma* the presence of gametes during all months of the year, although seasonal variability in egg number can occur (McCarthy *et al.*, 2003).

Studies of comparative gametogenesis can be helpful in elucidating and testing hypotheses about biogeography and the evolution of a taxon. Studies of oogenesis may be useful for generating phylogenetic hypotheses based on characteristics such as: (1) the presence, number, and location of definable ovaries; (2) the existence of extraovarian versus intraovarian oogenesis; (3) the release of previtellogenic oocytes into the coelom as solitary cells or in clusters; (4) mechanism of vitellogenesis; (5) the presence or absence of accessory cells; (6) the morphology of the egg envelope; and (7) the structure of the yolk pellet (Eckelbarger, 1988, 2006). In the present case, there are 12 genera and over 130 species of sabellariid worms (Read and Fauchald, 2012). Species of sabellariids can be solitary or gregarious and occur from continental shallow waters to continental shelf and slope depths. Morphological studies have elucidated the basal and derived genera within sabellariids (Capa *et al.*, 2012, Dales, 1952). Moreover, Capa *et al.*, (2012) have not found any phylogenetic relationship between some of the genera that construct colonies and reefs (e.g. *Phragmatopoma*, *Sabellaria*, *Gunnarea*).

Oogenesis has been studied in only a few *Phragmatopoma* and *Sabellaria* species and little progress in studies of sandcastle worm reproductive biology has been achieved in recent years (Culloty *et al.*, 2010). It would be most helpful if information about reproductive biology (Eckelbarger, 1988, 2006) was included in the recent phylogenetic of sabellariids (Capa *et al.*, 2012) and further studies should assess whether the pattern of oogenesis is consistent with previous phylogenetic studies published for the family.

The objective of this study is to describe the oogenesis of *Phragmatopoma caudata* from the southeast of the Brazilian coast using the histochemistry and transmission electron microscopy. For the first time, we provide a cytochemical description of oogenesis for Brazilian sabellariids. In addition, we compare consistencies in reproductive characteristics between South and North American *Phragmatopoma* spp. (Eckelbarger, 1979, Eckelbarger and Chia, 1978).

Material and methods

Sampling. *Phragmatopoma caudata* were collected at the Itararé beach at São Vicente, São Paulo State, Brazil (23°58'49"S; 46°22'02"W), during low tide in September 2009. Based on previous fieldwork data, it was known the specimens were carrying gametes in the sampling month, which is the beginning of the spring season (Faroni-Perez pers. obs.). In the laboratory, the specimens were removed from the sand tubes, anaesthetised by thermal shock with cold (4°C) sea-water and sexed. The length of the opercular crown (ventro-dorsal) was measured and only mature females were used, since the size ranged from 1.72 to 2.66 mm (Faroni-Perez, 2014).

Histology. Intact female worms (N=5) were fixed in 4% paraformaldehyde prepared with water from the sampling site containing sodium phosphate buffer 0.2 M (pH 7.2) for 24 hours at 4°C. After fixation, materials were rinsed in the same buffer (twice for 30 min), dehydrated in an ethanol series (70-95%), and embedded in methacrylate resin Leica®. Serial sections of 5 to 8 μm were obtained by Leica RM2252 microtome. Haematoxylin and eosin staining was proceeded according to Junqueira and Junqueira (1983) avoiding ethanol and xylene bath (Sant'Anna *et al.*, 2010) and used for traditional histological description. Histological images were acquired by a Leica DM2000 photomicroscope and digitised using the Leica IM50 software.

Cellular measurements. Cell measurements were obtained using the Leica IM50 software with appropriate system calibrations. All oocytes were measured using the 20X objective, and slides were stained by haematoxylin and eosin (HE). The largest cell diameter was obtained using only oocytes showing both a clear nucleus and a prominent nucleolus. Oocyte measurements were performed on five individuals with different operculum lengths and representing adult specimens. For each stage of oogenesis, the average oocyte diameters were obtained from ten cells per individual.

Histochemistry. Xylidine ponceau (Mello and Vidal, 1980) and mercuric-bromophenol blue staining techniques (Pearse, 1985) were used to demonstrate the presence of total protein. The Periodic acid-Schiff (PAS) technique was used to identify neutral polysaccharides with groups 1-2 glycol (Junqueira and Junqueira 1983; Pearse, 1985). The Alcian blue technique (pH 1.0 and 2.5) was chosen to demonstrate acidic polysaccharides (Junqueira and Junqueira, 1983), and Sudan Black B was used to determine the total lipids according Leica® protocol (Zara *et al.*, 2012).

Ultrastructure. For transmission electron microscopy (TEM), individual *Phragmatopoma caudata* (N=5) were fixed in 3% glutaraldehyde and 0.1 M (pH 7.3) sodium cacodylate buffer in filtered seawater (CBSF) for 4 hours and, post-fixed in 1% osmium tetroxide in the same buffer for 1 hour at 4°C. The "en bloc staining" was carried out using 1% aqueous uranyl acetate. The materials were dehydrated in ascending acetone series (50-100%) and embedded in Epon-Araldite resin. The ultrathin sections were stained with uranyl acetate and lead citrate and photographed in the Philips CM100 transmission electron microscopy at 80Kv electron beam.

Results

Histology and Histochemistry. The transverse dimension (ventral to dorsal) of the operculum of the analysed specimens ranged from 1.72 to 2.66 mm. The ovaries were clearly definable and closely associated to blood vessels of the intersegmental septa of *Phragmatopoma caudata* (fig. 1). In the ovary, the oogonia and oocytes were not surrounded by follicle cells (fig. 1). The oogonia, previtellogenic and early vitellogenic oocytes were attached to each other (fig. 1) until they were

released into coelom, where only late vitellogenic oocytes were observed (fig. 2).

The proliferative phase was marked by oogonia during mitosis, with a basophilic cytoplasm and an average diameter of 8.8 ± 2.2 μm. Three growth oocyte stages were classified: including oocytes in previtellogenesis (31.8 ± 5.3 μm) and early vitellogenesis (77.6 ± 9.4 μm), which were attached to the ovary, and late vitellogenesis that occurs in cells free in the coelom (100.4 ± 13.0 μm) (figs. 1 and 2).

The oogonia were smaller than the oocytes which were characterised by a large nucleus showing different stages of meiotic prophase and basophilic cytoplasm. Cell extensions connecting oogonia and blood vessels were noticeable (fig. 1). The previtellogenic oocytes showed a large nucleus and one or more nucleolus. Their cytoplasm was strongly basophilic without granules. Contrasting to the previtellogenic cell, the early vitellogenic oocytes were showed only one nucleolus in their large nucleus and the cytoplasm showed some acidophilic yolk granules (fig. 1). During late vitellogenesis, the mature or late vitellogenic oocytes occupied the entire coelomic cavity without direct contact to blood vessels (fig. 2). These oocytes were large and filled with yolk basophilic granules with less affinity to eosin than those in the previous stage (fig. 2). The basophilic vitelline envelope was clearly observed (fig. 2). There were no additional or follicular cells associated with these germ cells.

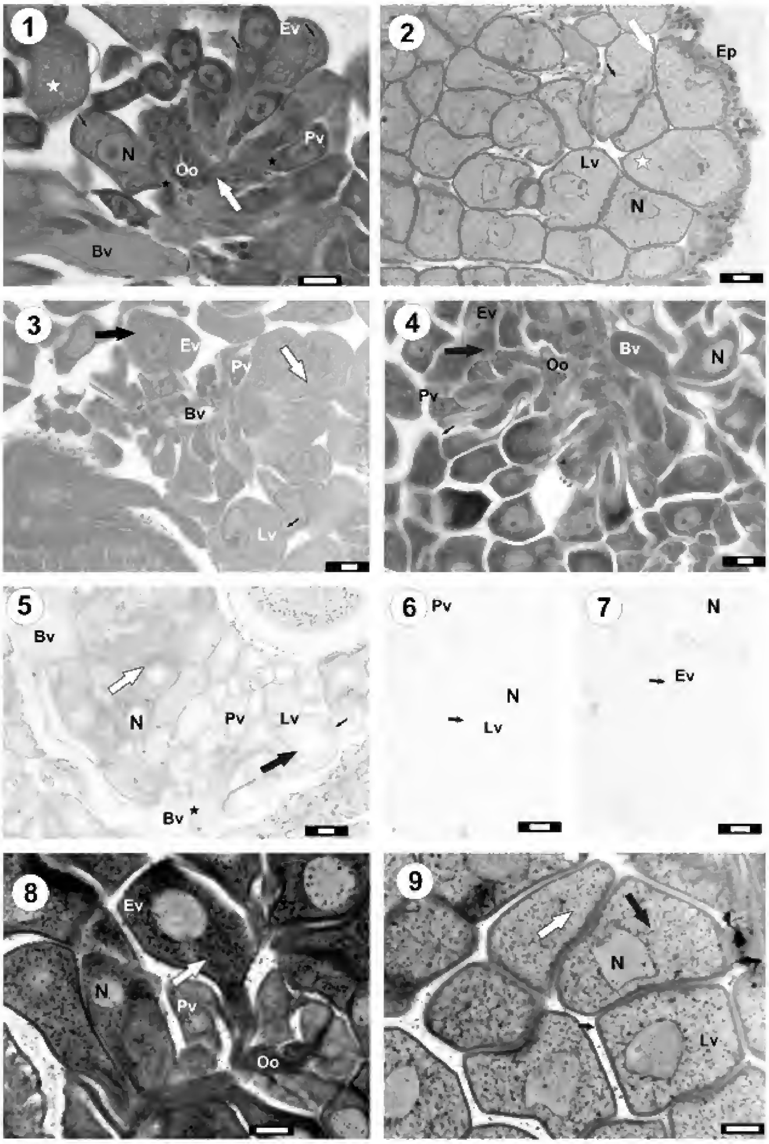
Histochemical analysis using xylidine ponceau (fig. 3) and mercuric-bromophenol blue (fig. 4) revealed that the cytoplasm of oogonia and previtellogenic oocytes were positively stained for proteins. During early vitellogenesis, the oocyte yolk granules, as well the egg envelope, were highly reactive compared to oocytes in late vitellogenesis (figs. 3 and 4). The oogonia were stained slightly for polysaccharides. However, the previtellogenic oocytes exhibited a slight cytoplasmic response to polysaccharides containing 1-2 glycol groups, such as glycogen (fig. 5). The oocytes in early vitellogenesis showed both cytoplasm and yolk granules that were slightly reactive to PAS. The oocytes in late vitellogenesis displayed a negative reaction to yolk granules and positive marks in the egg envelopes, indicating the glycoprotein constitution (fig. 5). The oogonia and oocytes at different stages were negative to Alcian blue tests (pH 1.0 and 2.5) for acidic polysaccharides (figs. 6 and 7). The oogonia were negative for lipid droplets, as stained by Sudan black B (fig. 8). In previtellogenic oocytes, some sparse lipid droplets were stained. The oocytes in early vitellogenesis showed qualitatively more lipid droplets than those in late vitellogenesis, which had several sparse lipid droplets. The yolk granules were negative to Sudan black B (fig. 9).

Ultrastructure

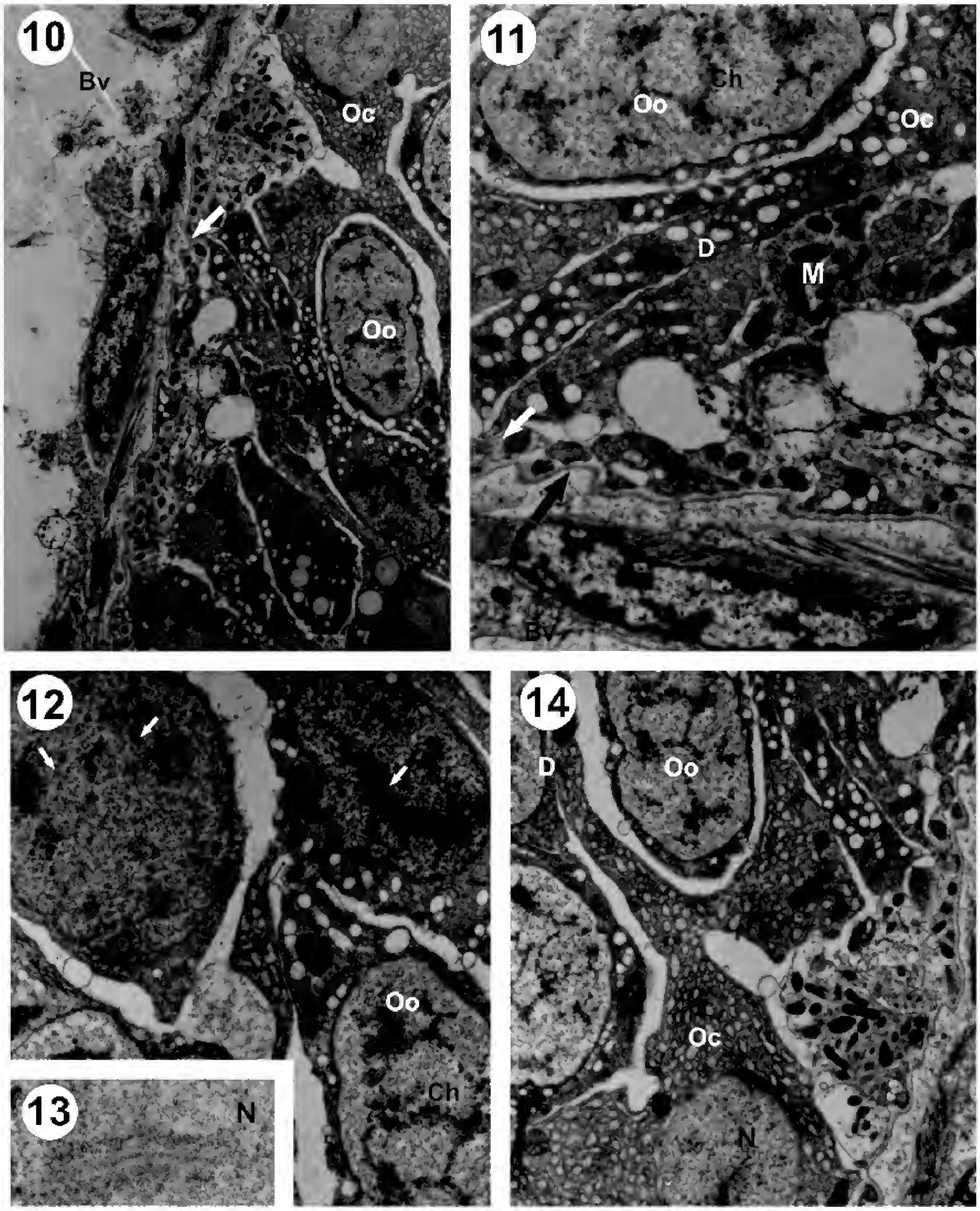
Proliferative phase. The ovary structure showed oogonia and oocytes. Oogonia connected to blood vessels via cellular prolongations to the flat endothelium (fig. 10). Between the germinative cells and the blood vessel occurred by a thin layer of connective tissue (figs. 10 and 11) showing collagen-like fibres contrasting with the electron-dense region of the ovary basal lamina (figs. 11). The oogonia, as well as the oocytes were connected via desmosomes (fig. 11). Differentiation between the oogonia and oocytes was indicated by the presence of mitotic chromosomes on an elliptical oogonia nucleus (figs. 10, 11, 12, and 14). The oocytes showed a rounded nucleolus, and the nucleoplasm contained dispersed chromatin and meiotic synaptonemal complexes (figs. 12-14). The oogonium cytoplasm was narrow and contained electron-dense mitochondria and a few vesicles of rough endoplasmic reticulum (RER) (figs. 10, 11, 12, and 14). The oocytes depicted had the same cytoplasmic characteristic, although cytoplasm was larger than in oogonia and had well-developed vesicular RER (figs. 10, 11, 14).

Growth phase. After meiosis, the previtellogenic oocytes increased both in cytoplasmic and nuclear volumes. The nucleus showed a single, large nucleolus and scattered heterochromatin blocks (figs. 15 and 16). Previtellogenic oocytes were elongated with a rounded, coelomic distal end. The plasma membrane maintained contact with other oocytes in prophase or previtellogenesis near the coelomic distal end (fig. 15). The cytoplasm was filled by RER consisting of parallel lamellae (fig. 16). A few electron-dense mitochondria, with shapes ranging from spherical to ellipitical, were common in the perinuclear cytoplasm (figs. 15-17). Accumulations of electron-dense α-glycogen were scattered in the cytoplasm (fig. 17), in agreement with the PAS-positive stains (fig. 5). The rounded end of the previtellogenic oocytes delineated a free margin in contact with the coelomic cavity characterised by the microvilli and thin egg envelope (figs. 18-21). The cortical cytoplasm showed many Golgi complexes, and several cortical granules were nearby (fig. 18). The cortical granules were filled with fibrous material of different electron densities (figs. 18-21). Among the small microvilli occurs the matrix of the egg membrane, which is granular while the basal region was electron-lucent and forming the wide perivitelline space at this phase (figs. 19-21). The microvilli apex, above the egg envelope, showed an expansion bearing extensive filamentous adornment with an electron-dense central region (figs. 19 and 20). The end of the previtellogenesis was determined by the beginning of endocytotic activity marked by pits in the plasma membrane and presence of coated vesicles in the cortical cytoplasm (figs. 20 and 21) at the same time that the yolk granules arose.

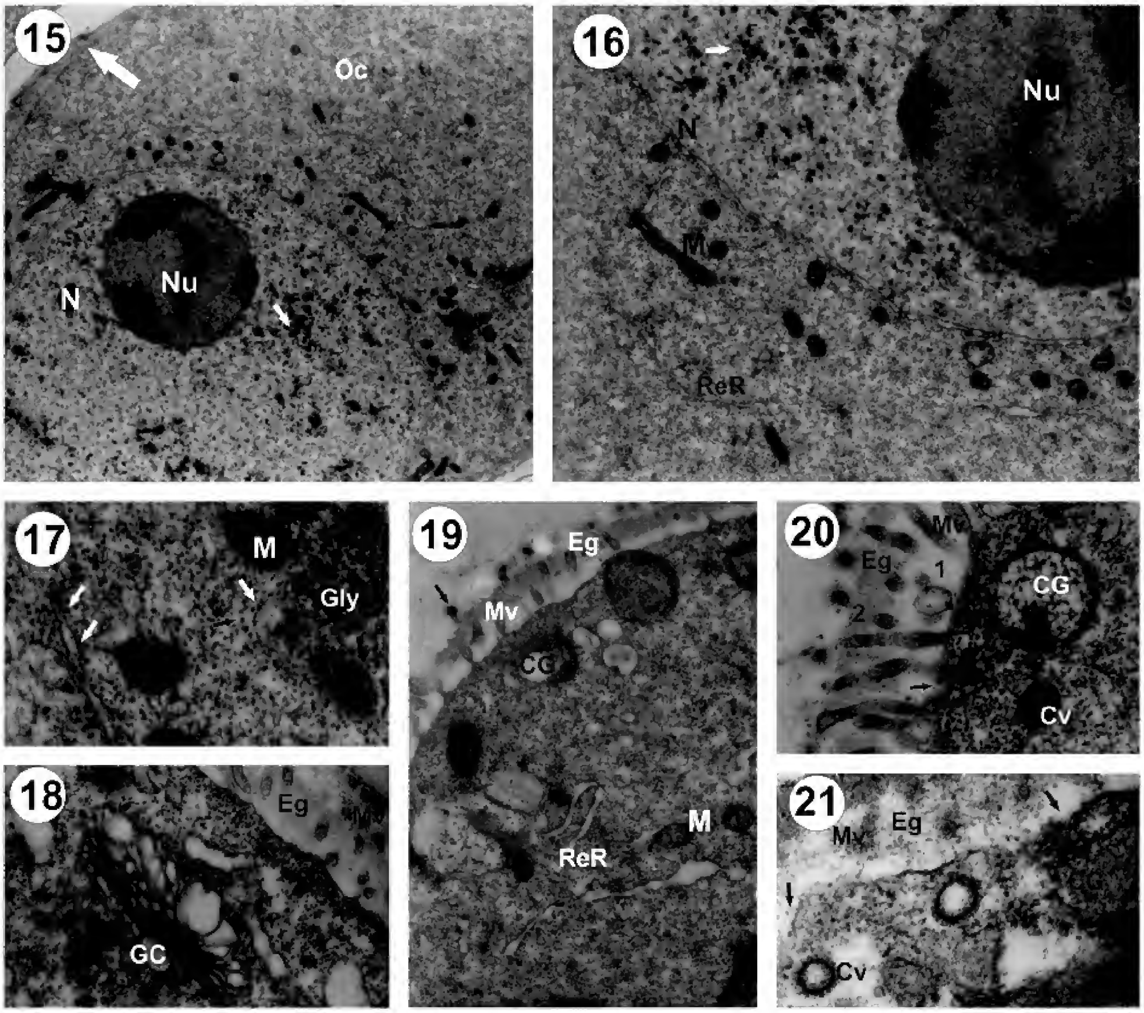
During vitellogenesis or exogenous phase of yolk production, two distinct ultrastructural oocyte stages were observed (*i.e.* oocytes in early and late stage of vitellogenesis). During early vitellogenesis, the oocytes were attached to the ovarian blood vessel and the nucleus showed the same characteristics as the previtellogenic oocytes, with many scattered heterochromatin blocks (figs. 22 and 23). The large number of nuclear pores were indicative of the high nuclear activity during the early vitellogenesis (fig. 23). Clusters of granular electron-dense material, or nuages, were observed next to the nuclear envelope and perinuclear cytoplasm (fig. 23). The cytoplasm was filled with lamellar rough ER, several lipid droplets, and small yolk granules (figs. 22-25). The yolk granules were rounded, compact and showed areas with varied electron densities (figs. 24 and 25). Inside the yolk granules, some electron-lucent spherical areas were visible. (fig. 24). Particles of α-glycogen were adjacent to the yolk granules (fig.



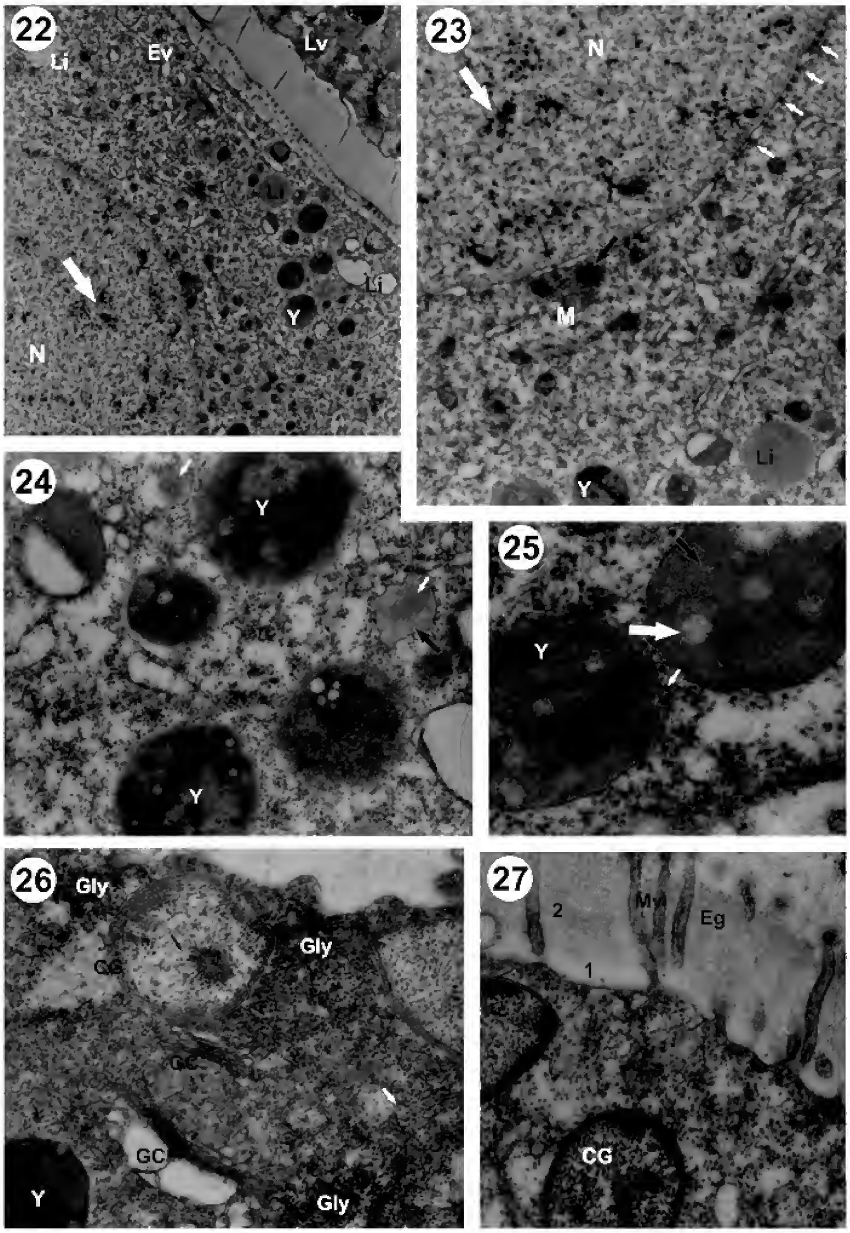
Figures 1–9. Histology and Histochemistry in *Phragmatopoma caudata* of the SW Atlantic. Figures 1 and 2 Oogonia (Oo) and previtellogenic oocytes (Pv) of early vitellogenesis (Ev) associated with the intersegmental blood vessels (BV) for cytoplasmic prolongations (black star). Oocytes in advanced and late vitellogenesis are released into the coelomic cavity (white star). Oocytes in vitellogenesis have intensely acidophilic granules, and the ripe oocytes are less acidophilus (black arrows). Figure 2 depicts oocytes in late vitellogenesis occupying the entire coelom with a basophils vitelline envelope (white arrow). N = nucleus; H&E staining. Scales = 20 μm. **Figures 3 and 4** Techniques used to visualize basic and total proteins, respectively. The previtellogenic and vitellogenesis (Ev) oocytes (Pv) have intense granules (large black arrow), and the ripe in late vitellogenesis (Lv) are less reactive (white arrow). The vitelline envelope is reactive to protein (small arrow). Scales = 20 μm. **Figure 5** Technique for visualizing neutral polysaccharides with positive staining in both the previtellogenic oocyte cytoplasm and the vitellogenic oocyte granules. The reactivity disappears in the vitellogenic granules of ripe oocytes, but surrounding these is a noticeable positive staining (large black arrow). Star = oogonia with little reactivity to neutral polysaccharides. The egg envelope is reactive to PAS (small arrow). Scale = 20 μm. **Figures 6 and 7** Absence of acid polysaccharides (pH 1.0 and 2.5), respectively, in oogonia and oocytes in *P. caudata*. Scales = 20 μm. **Figures 8 and 9** Lipids stained by Sudan Black B. The oogonia are uniformly positive (small white arrow), while the oocytes during previtellogenesis and vitellogenesis have droplets in the cytoplasm (large black arrow). The yolk granules are reactive during vitellogenesis, while the staining is less intensive (large white arrows) in the mature oocytes. The vitelline envelopes have lipids (small black arrow). Scales = 20 μm.



Figures 10–14. Ultrastructure in *Phragmatopoma caudata* of the SW Atlantic. Proliferative Phase. Figures 10 and 11 Oogonia (Oo) and oocytes (Oc) connected via cellular prolongations (white arrow) to the endothelium of the intersegmental blood vessel (Bv). Note that the contact of ovary basal lamina is quite electron dense (black arrow). Desmosomes (D) form the oocyte-oocyte junction (Figure 10, 1,150X; Figure 11, 2,050X). M = mitochondria; Ch = chromosome. **Figure 12** Oogonia with mitotic chromosomes (Ch) and oocytes in prophase with finely granular chromatin and chromosomes united by synaptonemal complexes (arrows) (2,400X). **Figure 13** Synaptonemal complex (33,600X). N = nucleus. **Figure 14** Narrow cytoplasm in oogonia (Oo) androunded nucleus. Oocyte (Oc) with wide cytoplasm and adhesion via desmosomes (D) (2,050X).



Figures 15-21. Ultrastructure in *Phragmatopoma caudata* of the SW Atlantic. Grow Phase. Figures 15 and 16 Previtellogenic oocyte with a large nucleus (N) and nucleolus (Nu), as well as small rough heterochromatin clumps (small arrow). The cytoplasm is filled with rough, lamellar endoplasmic reticulum (RER) and noticeable mitochondria (M) in the perinuclear cytoplasm. The elongated portions of these cells create adhesion with adjacent oocyte (large arrow) (Figure 15, 2,050X. Figure 16, 4,200X). Oc = oocyte. **Figure 17** Cytoplasm showing glycogen α (Gly) greater than the ribosomes (white arrows) attached to the reticulum (black arrow) (13,500X). M = mitochondria. **Figures 18 and 19** Cortical cytoplasm of the rounded surface, showing Golgi complexes (GC) close to the cortical granules (CG), positioned beneath the microvilli (Mv) with the expansion bearing extensive filamentous adornment above the vitelline envelope (Eg) (Figure 18, 10,500x. Figure 19, 5,400X). **Figures 20 and 21** Plasma membrane on a rounded surface showing endocytic depressions (black arrow) and coated vesicles (Cv). The vitelline envelope is composed by means of medium-apical extracellular matrix (2) in relation to microvilli (Mv). The basal region is electron-lucent and forming the beginnings of the perivitelline space (1) (Figure 20. 13,500X. Figure 21, 28,000X). GC = Golgi complexes, Eg = vitelline envelope.



Figures 22-27. Ultrastructure in *Phragmatopoma caudata* of the SW Atlantic. Grow Phase. Figure 22 Oocyte during early vitellogenesis (Ev) with attributes of several yolk granules (Y) and lipid droplets (Li) in the cytoplasm, adjacent to an oocyte in late vitellogenesis (Lv) which vitelline envelope (Eg) thick (1,450X). N = nucleus, arrow = clumps of heterochromatin. **Figure 23** Nucleus showing the heterochromatin clumps (large white arrow) and many complex pores in the nuclear envelope (small white arrows). The perinuclear cytoplasm aspect of granular material accumulations, juxtaposed with the nuclear envelope (black arrow) and next to mitochondria (M) (2,050X). **Figure 24** Small and rounded yolk granules (Y) with different electron-densities and lucid spheres are observed inside. Besides Vesicles containing some electron-dense material (white arrow) also have lucid spheres (black arrow) and resemble nascent yolk granules (8,200X). **Figure 25** Yolk granules (Y) of oocytes at the beginning of vitellogenesis showing varied electron-densities (black arrow) and electron-lucent spheres (large white arrow). Glycogen is also noticed (small white arrow) (21,500X). **Figure 26** Cortical cytoplasm showing Golgi complexes (GC) close to the cortical granules (CG), which are filled with a fibrous material (black arrow). Note the large amount of glycogen (Gly) in the cytoplasm (1,150X). White arrow = glycogen. **Figure 27** Microvilli (Mv) are larger and the egg envelope (Eg) thicker (2), relative to the previous stage. The perivitelline space (1) appears thinner (1500X).

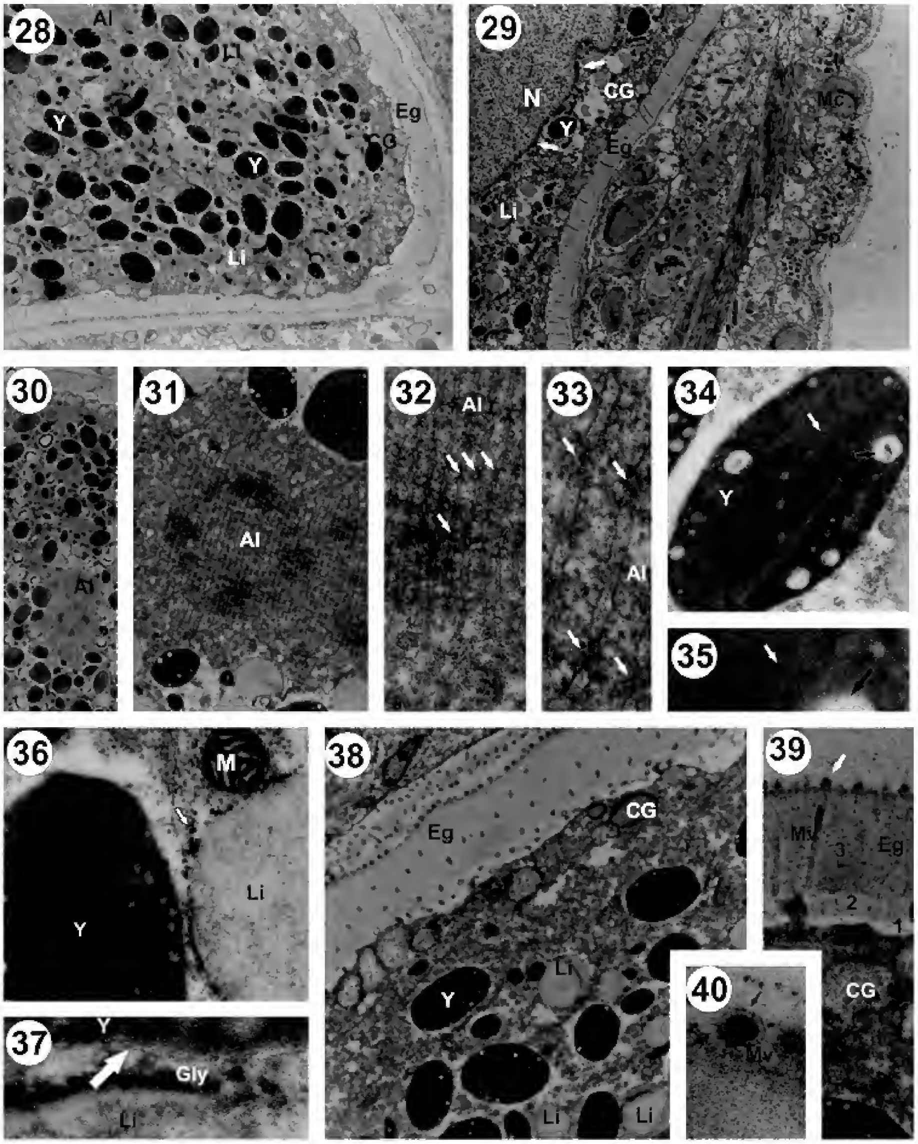
Table 1. Ultrastructural oogenesis in *Phragmatopoma* spp. from Northwest and Southwest Atlantic Ocean.

	NW Atlantic*	**SW Atlantic****
Anterior face of septal blood vessel ciliated	present	present
Oogenesis (type)	intraovarian	intraovarian (until early vitellogenesis) and extraovarian (during late vitellogenesis)
Follicle cells	present	absent
Peritoneal capsule covering ovary during vitellogenesis	present	absent
Asynchronous oogenesis	present	present
Amoeboid cells	present	absent
Mitochondrial cloud locality (in previtellogenic oocytes)	one part of oolema	perinuclear cytoplasm
Golgi complexes	adjacent to oolema where microvilli are formed	close to cortical granules
Golgi complexes (arrangement)	semicircle	parallel
Cortical granules	early vitellogenesis	previtellogenesis
Endocytosis	present	present
Coated vesicles	present	present
Annulate lamellae (coelomic eggs)	present	present
Golgi complexes (coelomic eggs)	few	few
Egg mambrane formation	early vitellogenesis	previtellogenesis
Egg envelope with extracellular matrix (coelomic eggs)	present	present
Microvilli with granular tips	present	present
Intermicrovillar distance change during oogenesis	NA	present
Granules of microvilli changes complexity and number increased during oogenesis	present	present
Autosynthetic crystallized yolk granules (coelomic eggs)	synthesis begin prior to the heterosynthetic yolk granules	synthesis begin after to the heterosynthetic yolk granules
Golgi complexes where occur the synthesis of heterosynthetic yolk granules	absent	present

* Eckelbarger and Chia 1978; Eckelbarger 1979 ** This study. NA: no information available.

25). The glycogen was abundant throughout the cytoplasm, and its size was large compared to the ribosomal particles (fig. 26). The remainder of the cytoplasm had the same characteristics as the previtellogenic stage. The Golgi complexes were more common in the cortical cytoplasm near the cortical granules and a large number of mitochondria were observed (figs. 22-27). The endocytic activity was observed in the plasma membrane, and the larger microvilli maintained the same apical characteristics as observed at the end of previous stage. The perivitelline space (electron-lucent) was smaller and the vitelline envelope thicker than observed for the previtellogenic oocytes (fig. 27).

The oocytes in late vitellogenesis (*i.e.* ripe) were filled with yolk granules, lipid droplets and thick vitelline envelope (fig. 28). These oocytes occupied the entire coelomic cavity toward the body wall (figs. 29). The remarkable cytoplasmic structure was the annulate lamellae that formed patches through the yolk and lipid droplets (fig 28 and 30). The annulate lamellae were characterised by several fenestrated endomembranes with parallel structures (figs. 31-33). The yolk granules were distinct to the early of vitellogenesis forming an elliptical and extremely compact structure. These mature yolk granules showed a medullar crystalline substructure and a cortex with electron-lucent spheres (figs. 34-36). These granules were surrounded



Figures 28-40. Ultrastructure in *Phragmatopoma caudata* of the SW Atlantic. Growth Phase. Figure 28 Oocyte during late vitellogenesis filled with yolk granules (Y) and lipid droplets (Li). Note the cytoplasmic patches where the annulate lamellae (Al) are located and the well-developed egg envelope (Eg) (810x). **Figure 29** Ripe oocytes occupy the entire coelomic cavity toward the body wall (810x). MC = muscle; Ep = epidermis. **Figure 30** General view of the annulate lamellae (Al) between yolk granules and lipid droplets (560X). **Figures 31-33** The annulate lamellae are composed by fenestrated endomembranes (arrows) parallel to each other arranged (Figure 31, 2,900X. Figure 32, 10,500X. Figure 33, 3,100X). **Figure 34** Mature yolk granules with an elliptical shape (Y) showing a medullar crystalline substructure (white arrow) and non-crystalline cortex with electron-lucent spheres (black arrow) (13500X). **Figure 35** Crystallised medullar in a parallel arrangement (white arrow) and a cortical lucid sphere (black arrow) (43,000X). **Figure 36** Yolk granules (Y) and lipid droplets (Li) in the cytoplasm, with the presence of glycogen granules (arrow) close to the droplets (13,500X). M = mitochondria. **Figure 37** Details of yolk granules delimited by the membrane (arrow) next to a lipid droplet (Li) and glycogen (Gly) (61000X). **Figure 38** Cortical cytoplasm filled with yolk granules (Y), lipid droplets (Li) and cortical granules (CG) below the plasma membrane. Notice the thick egg envelope (Eg) (2,050X). **Figure 39** The perivitelline space (1) is narrow and the egg envelope (Eg) is composed of two layers (2 and 3) with different electron-densities. The winding microvilli (Mv) are completely immersed in the egg envelope. Only the apical surface of the microvilli is in contact with the coelomic cavity (arrow) (5,400X). CG = cortical granules. **Figure 40** Microvilli apex showing the expansion bears extensive filamentous adornment with an electron-dense centre (31,000X).

by membrane since the previous phase (fig. 37). The endoplasmic reticulum and a few Golgi complexes were more common at the cortical cytoplasm. The cytoplasm showed fewer glycogen granules, particularly around the yolk granules and lipid droplets (figs. 36 and 37). The oocyte surface had long, sinuous microvilli sharing similar characteristics to the other phases. Additionally, endocytic pits were observed. The microvilli were extensive filamentous adornment structures on the outer surface of oocytes and in the coelomic cavity. The egg envelope displayed two different electron-dense layers and a very thin perivitelline space (figs. 38-40).

We found several differences in oogenesis among the Northwest and Southwest Atlantic Ocean populations, and the concise ultrastructural descriptions are summarised in Table 1.

Discussion

The histochemical results presented here are novel for the Sabellariidae. Oogenesis in *Phragmatopoma caudata* from São Vicente, SP, Brazil, entails a number of new features: 1) a distinct ovarian proliferation characterised by small oogonia clusters that were connected by intercellular extensions to the blood vessel walls; 2) an absence of accessory, or follicular, cells; and 3) a complex vitellogenesis cycle. There were also different stages and mechanisms of yolk formation and yolk precursors from the circulatory system, endocytic activity in previtellogenic oocytes, and the crystallisation in vitellogenic oocytes.

Since oogenesis in *Phragmatopoma* from the southwestern Atlantic Ocean has never been reported, it has not been possible until now to consider intraspecific variation in terms of reproductive characteristics. Oogenesis is different between the worms in the SW Atlantic (present results) from those in the NW Atlantic (Eckelbarger, 1979, 1983, 2005, 2006). The heterogeneity revealed in this study suggests additional molecular analysis should be carried out to investigate the current synonymy based on morphological analyses (Dos Santos *et al.,* 2011, Kirtley, 1994). The differences in oogenesis identified between these geographically remote populations may reflect an evolving divergence among the populations.

The nature of oocyte development (*i.e.* from autosynthetically to heterosynthetically) reported in our study differs from those described in NW Atlantic worms by Eckelbarger (1979). Oocyte development in *P. caudata* from the SW Atlantic occurs after the dissemination of the previtellogenic oocytes into the coelom in a free-floating phase. This mechanism of oogenesis is an extraovarian oogenesis process (Eckelbarger, 1983, 1994, 2006). The absence of distinct ovaries in the peritoneum also occurs in some species of nereidids, phyllodocids, and sphaerodorids (Olive, 1983). In these cases, the germ cells, oogonia or very early oocytes, are realesed into the coelom. In addition, in species of pectinariids and sabellids solitary previtellogenic oocytes are released into the coelom where they undergo vitellogenesis (Eckelbarger, 2005).

Both autosynthetic and heterosynthetic mechanisms of yolk production could be observed during vitellogenesis in specimens from the Southern Hemisphere as had previously been described for NW Atlantic specimens (Eckelbarger, 1979). Nevertheless, differences in the cytology of vitellogenic process were observed between worms from the two populations. In NW Atlantic worms, only a single pellet is formed by either mechanism producing two morphotypes of yolk granules (Eckelbarger, 1979). On the other hand, vitellogenesis appeared to be a combination of both processes in the SW Atlantic worms and a single morphological type of yolk granule is formed. In *Phragmatopoma lapidosa* (syn. *P. caudata*), the type II yolk granules, formed heterosynthetically from endocytosis of yolk precursors from the blood vessel, appear much later than type I, which are formed autosynthetically (Eckelbarger, 1979). Although the two morphological types of yolk were described for NW Atlantic worms, the author highlighted that it is possible precursors are sequestered endocytotically from the circulatory system assembly into type I yolk bodies. The convoluted contacts between blood-vessels and developing oocytes (early developing oocytes) increase the surface area (Olive, 1983) and may optimize the uptake of nutrients and yolk precursors from blood vessels. Furthermore, presumably the single type of yolk observed in worms from the SW Atlantic may be the result of the earlier appearance of coated pits besides the oocyte releasing to the coelom before vitellogenesis is completed. In *P. lapidosa* (syn. *P. caudata*) from the NW Atlantic, as well as in *P. caudata* from the SW Atlantic, the ovaries are ephemeral, repeated in a large number of segments, and have the centres of germ cell proliferation connected to blood vessels via the intersegmental septa (Eckelbarger, 1979, 2005, 2006, Eckelbarger and Chia, 1978). The mitotic divisions in *P. caudata* were detected only in the germ cells during the intraovarian growth phase associated with blood vessels.

Follicle cells. In contrast to *Phragmatopoma caudata* from the SW Atlantic population, worms from the NW Atlantic had follicle cells and oocytes connected to the blood vessels during the complete oocyte maturation. Consequently, the vitellogenesis occurred inside the ovaries which, were covered by a peritoneal capsule (Eckelbarger, 1979, Eckelbarger and Chia, 1978). Although lacking physiological evidence that material actually passes from the follicle cells to the developing oocytes, the follicle cells of *Phragmatopoma lapidosa* (syn. *P. caudata*) appear to serve as intermediaries between the oocytes and the surrounding coelomic fluid (Eckelbarger, 1979). Oocytes surrounded by a distinct follicle cells layer as described in *P. lapidosa* (syn. *P. caudata*) species of the NW Atlantic (Eckelbarger, 1979) are absent in *P. caudata* from the SW Atlantic. Thus, the nutrient sources for yolk precursors provided by follicle cells, and amoeboid cells in the NW Atlantic species are directed uptake from the blood vessel (early vitellogenesis) and coelom to proteosythesis for *P. caudata* from the SW Atlantic. In polychaetes, little is known about protein synthesis during oogenesis (Lee, 1988; Song and Lee, 1991). In addition, gene expression connected with oogenesis remains unstudied and therefore nothing is known of which genes code for follicle cells and how differentiation-specific proteins are involved in the formation of the ovarian follicular cells or the timing of oocytes to be released to the coelom among close related species. A prerequisite to further analysis of the role of genes coding for

follicle cells is to find a model to assess how small changes in the genetic structure might affect the adhesion and interrelationships between different cell types. Since current findings reveal morphological variation in the patterns of oogenesis in the two populations described so far, we recommend *Phragmatopoma* as a promising model for further molecular analysis.

Endocytosis and Yolk Synthesis. The presence of coated vesicles in the cortical cytoplasm after the end of previtellogenesis and during the whole vitellogenesis confirms the heterosynthetic process of yolk granules formation. In worms from the SW Atlantic, the large number of coated vesicles observed on the surface of oocyte indicated a common mechanism of substance uptake, primarily the extrinsic vitellogenesis proteins. In addition, the large numbers of coated vesicles indicated the high endocytic activity and relatively short vitellogenesis period (Eckelbarger, 1983). Oogenesis occurs quickly in *P. lapidosa* (syn. *P.caudata*) from the NW Atlantic, and hundreds of coated vesicles were seen for each oocyte during vitellogenesis (Eckelbarger, 1979, 1983) similar to the observations in this study.

Endocytosis is known as a distinct mechanism for incorporating large molecular weight exogenous yolk proteins into the oocyte and there may be some association between the number of endocytotic pits generated along the oocyte oolemma and the length of the vitellogenic phase (Eckelbarger, 1980, 1983). In *Phragmatopoma lapidosa* (syn. *P. caudata*) from the NW Atlantic, precursor molecules for yolk formation via an autosynthetic process may enter the oocyte through the microvillus, that appears just prior to vitellogenesis by means of combined efforts of Golgi complexes and RER assembled into yolk bodies (Eckelbarger, 1979). Even so, the autosynthetic and heterosynthetic processes of yolk synthesis were similar in *Phragmatopoma* worms from both the NW and SW Atlantic Ocean populations. On the other hand, in *Phragmatopoma caudata* from the SW Atlantic, the extraovarian oogenesis occurs during late vitellogenesis without involving follicle cells as reported for other Sabellariidae. Thus, our results provide additional evidence that oogenesis in the populations of the NW and SW Atlantic is clearly dissimilar to previously described (Eckelbarger, 1979, 1983, 1984, 2005). A comparative study has revealed differences in oogenesis among four *Capitella* species (Eckelbarger and Grassle, 1983). The variation in abundance and relative size of specific yolk pellets in the eggs of *Capitella* spp. was apparently related to the quantities of lipid and glycogen stored in the follicle cells. It is plausible that such differences have a significant impact on embryogenesis and larval development.

Cortical Granules. The population from the SW Atlantic Ocean displayed Golgi complex activity, synthesis of cortical granules near the plasma membrane and yolk precursors were uptake from the circulatory system. The cortical granules showed different electron densities and fibrous material similar to that observed in worms from the NW Atlantic (Eckelbarger, 1979, 2005). In *Phragmatopoma lapidosa* (syn. *P. caudata*) from the NW Atlantic the cortical granules appeared early in vitellogenesis, our results showed earlier appearance, during previtellogenesis.

Annulate lamellae. The annulate lamellae are cytomembranes containing pores and are frequently attached or connected to the endoplasmic reticulum. Commonly, ribosomes are attached to the membranes that extend from the annulate lamellae (Kessel, 1989). In polychaetes, both ooplasmic and internuclear annulate lamellae have been described in some species but their function is unknown (Eckelbarger, 1988). In oocytes of *Phragmatopoma caudata* from the NW Atlantic, the annulate lamellae appear during the mid-stage of vitellogenesis. In late-stage, the annulate lamellae are still observed in the ooplasm (Eckelbarger, 1979), similar to this study. Although its function is unclear, in *P. caudata* from the SW Atlantic, the annulate lamellae is closely associated with yolk granules and lipid droplets, and persists until the oocyte is fully-grown.

Egg envelope. Ultrastructural results reveal that the oocyte surfaces in *Phragmatopoma caudata* from the SW Atlantic population and in *P. lapidosa* (syn. *caudata*) from the NW Atlantic population (Eckelbarger and Chia, 1978) have a granular extracellular matrix layer. The egg envelope of both *Phragmatopoma* populations exhibited changes during oocytes maturation. In worms from the NW Atlantic, the microvilli appear during the early growth phase, and related granules are continuously produced. The following phase is characterised by an increase in granule formation by the existing microvilli which are no longer being formed (Eckelbarger and Chia, 1978).

In *Phragmatopoma caudata* from the SW Atlantic population, the glycoproteinaceous egg envelope, composed only of neutral polysaccharides, is a layer of granules whose complexity and size increase during vitellogenesis and may play a role as a selective barrier for nutrient uptake. Oocytes in contact with coelomic fluid might induce an increase in surface area through elaboration of the oolemma into numerous microvilli showing expansion. This may facilitate the uptake of low molecular weight yolk precursors (Eckelbarger, 1988). However, the differences observed in the formation of the egg membrane between the NW and SW Atlantic Ocean populations may indicate early stages of genetic divergence, or alternatively, may be an adaptive response to the mechanisms underlying the local environment.

Although a recent study supported the synonymy of *Phragmatopoma* species (Dos Santos *et al.*, 2011), this action could be premature (Capa *et al.*, 2012). The plasticity found in oogenesis among the worms from Florida, USA, and São Paulo, Brazil, are considerable (Table 1). In addition we should not assume that the reproductive traits reported in the literature for gregarious intertidal populations of sabellariids would necessarily be accurate for the entire family, including those solitary deep-sea species. Our work demonstrates asynchronous oogenesis in *Phragmatopoma caudata* which was similar to that found in *P. lapidosa* from the NW Atlantic (Eckelbarger, 1979). Thus, different stages of oocyte development can be observed simultaneously within a single organism. This contrasts with *Sabellaria alveolata* in which a gametogenesis is synchronous and all oocyte are in the same stage during the reproductive cycle (Culloty *et al.*, 2010). However, in *S. alveolata* populations from the NE Atlantic, the various gametogenesis stages also occurred simultaneously among

individuals in the population (Culloty *et al.,* 2010). The factors regulating the onset of the reproductive cycle and spawning events are poorly understood in Sabellariidae worms.

In conclusion, oogenesis observed in *Phragmatopoma caudata* of the SW Atlantic is similar to that found in the NW Atlantic indicating that the taxa are closely related and recently separated. However, the diverse aspects of oogenesis documented here give support to the reproductive plasticity among the geographically remote populations. We suggest that the taxonomic status be reviewed incorporating additional traits. Thus, heterogeneity in both oogenesis and oocyte development patterns among the worm populations from the Northern and Southern Hemisphere may indicate (1) different species, and (2) differences in the production of ovarian oocytes due to latitude (i.e. environmental drivers). Further studies, using broad latitudinal comparisons of oogenesis and molecular analyses along with descriptions of the ultrastructure of sperm, are required to determine the number of possible species. It would then be possible to determine if the geographically remote worm populations with their heterogeneous characteristics are the evolutionary products of distinct past tokogenetic events.

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Character mapping and cladogram comparison versus the requirement of total evidence: does it matter for polychaete systematics?

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Abstract

Fitzhugh, K. 2014. Character mapping and cladogram comparison versus the requirement of total evidence: does it matter for polychaete systematics? *Memoirs of Museum Victoria* 71: 67–78.

The practice of partitioning data for the inferences of phylogenetic hypotheses has become a routine practice in biological systematics. Two popular approaches: (i) mapping 'morphological' characters onto 'molecular' phylogenies, and (ii) comparing 'morphological' and 'molecular' phylogenies, are examined in light of what is known as the requirement of total evidence. Inferences of phylogenetic hypotheses, indeed all taxa, occur by a type of non-deductive reasoning known as abduction. The intent of abduction is to offer at least tentative causal accounts that explain character data. The rational acceptance of abductively derived hypotheses is subject to conditions of the requirement of total evidence as a matter of the evidential support for those hypotheses. It is shown that both character mapping and comparisons of cladograms using partitioned datasets are procedures that severely reduce the credibility of phylogenetic hypotheses. This problem is alleviated by acknowledging the formal structure of the why-questions we ask in relation to character data, for which phylogenetic hypotheses serve as answers.

Keywords abductive inference, biological systematics, cladograms, phylogenetic hypotheses, requirement of total evidence

"The requirement of total evidence is not itself controversial." (Kelly, 2008: 64)

Introduction

Biological systematics has entered a state of complacency, where research agendas tend to follow prescribed methodological rules that satisfy requirements for using particular software packages or programs that lead to phylogenetic (or otherwise) hypotheses, or claim to provide empirical assessments of those hypotheses. This state of affairs might be expected if we adhere to Kuhn's (1970) notion of 'normal science' (but see Popper, 1970). Regardless of the consensus that might obtain in a field of science, this does not afford the accepted protocols and methods immunity from critique. There is, for instance, the expectation that scientific inquiry operates within the constraints set by the basic principles of rational reasoning (Williamson, 2000; Thagard, 2004), where the acceptance of propositions is governed by evidential support. That support comes either in the form of evidence leading to inferences of hypotheses and theories or the subsequent evidence supplied during empirical testing. If approaches to inquiry agreed upon among a group of scientists are identified as leading to less than rational conclusions due to the exclusion of evidence, either during the formulation or testing of hypotheses/theories, then the intended goal(s) of such inquiries and associated methods must be judged relative to the criteria that determine the credibility of those hypotheses/theories. Whitehead's (1925: 18) admonition remains relevant: "The progress of biology and psychology has probably been checked by the uncritical assumption of half-truths. If science is not to degenerate into a medley of *ad hoc* hypotheses, it must become philosophical and must enter into a thorough criticism of its own foundations."

Among presentations at the 11th International Polychaete Conference that addressed phylogenetic relationships, the most common approach was that of 'character mapping'. Phylogenetic hypotheses, implied by cladograms, are inferred for sets of sequence data, and via those diagrams various conclusions are drawn regarding the evolution of morphological traits (cf. Halanych et al., 2001; Bleidorn et al., 2003; Hall et al., 2004; Halanych, 2005; Rousset et al., 2006; Schulze, 2006; Struck et al., 2007; Colgan et al., 2008; Kupriyanova and Rouse, 2008; Wiklund et al., 2008; Vrijenhoek et al., 2009; Zanol et al., 2010; Struck et al., 2011; Magesh et al., 2012; Goto et al., 2013). Interestingly, the inverse—obtaining transformation series via the mapping of nucleotides on cladograms inferred from 'morphological' characters—is never considered. An equally widespread approach involves comparisons of cladogram topologies inferred from different datasets for the same group of organisms (cf. Rousset et al., 2003, 2004; Eeckhaut and

Lanterbecq, 2005; Halanych, 2005; McHugh, 2005; Kupriyanova et al., 2006; Sperling et al., 2009; Zrzavý et al., 2009; Parry et al., 2014). The popularity of character mapping and cladogram comparisons is by no means limited to polychaetes, as perusals of such journals as *Molecular Phylogenetics and Evolution*, *Nature*, and *Systematic Biology* will attest. Regardless of their popularity, the problems surrounding these techniques are so significant as to preclude their use. This paper will identify the epistemic difficulties in light of the necessary principle of rationality known as 'the requirement of total evidence'.

Why systematics?

Determining that protocols such as cladogram comparisons and character mapping are problematic requires that we first acknowledge the intent of reasoning in biological systematics. The overarching goal of scientific inquiry is to acquire causal understanding of the phenomena we observe/describe, which also affords opportunities for predictions into the future (Hempel, 1965; Hanson, 1958; Rescher, 1970; Popper, 1983, 1992; Salmon, 1984a; Van Fraassen, 1990; Strahler, 1992; Mahner and Bunge, 1997; Hausman, 1998; Thagard, 2004; Nola and Sankey, 2007; de Regt et al., 2009; Hoyningen-Huene, 2013). As a field of science, we should expect the objective of systematics to be consistent with that of other fields. The consequence is that the aim of systematics is to causally account for the differentially shared characters we observe among organisms, whether extant or represented as fossils (Fitzhugh, 2012, 2013, and references therein). Consider the actions of compiling observation statements into a data matrix and 'inferring cladograms.' The implied intent would have to be that of explaining, by way of past evolutionary events, differentially shared characters. The primacy of explanation in systematics is, however, rarely cogently articulated and has led to a tendency to only focus on the diagrammatic qualities of cladograms, 'phylogenies' or 'trees', with inordinate attention on 'groups' and topologies, rather than recognising that cladograms are composite hypotheses representing at least three classes of causal events: (i) character origin/fixation among individuals of reproductively isolated ancestral populations and (ii) subsequent population-splitting events (Fitzhugh, 2012: Figs 1, 4; 2013: Fig. 1), as well as (iii) species hypotheses, which are inferred prior to cladograms-as-hypotheses, denoting more proximate accounts of character origin/fixation among individuals of reproductively isolated populations observed in the present. Causal events (i)–(iii) are implied by the 'interior branches', 'nodes' and 'terminal branches', respectively, that make up cladograms. Needless to say, cladograms typically convey nothing in the way of specifics regarding the causal events they are intended to imply. There are additional classes of hypotheses utilised in systematics (cf. Hennig, 1966: Fig. 6; Fitzhugh, 2012: Table 1; 2013: Table 1), but the emphasis in this paper will be on those that are phylogenetic. Presenting a diagram as a 'phylogeny' minimally assumes that it causally accounts for specifiable characters that were the basis for the inference, e.g. a data matrix. To assert that cladograms do not have to meet such an obligation would reduce them to nothing more than rhetorical devices with little or no scientific utility.

Phylogenetic reasoning

Acknowledging cladograms, trees, phylogenies, etc., as sets of explanatory accounts providing at least initial causal understanding of select characters of organisms necessitates that we identify the particular type of reasoning employed to move from observation statements, as data matrices *partim*, to cladograms. Inferring tentative causes from observed effects is known as abductive reasoning, or abduction (Peirce, 1878, 1931, 1932, 1933a, 1933b, 1934, 1935, 1958a, 1958b; Hanson, 1958; Achinstein, 1970; Fann, 1970; Reilly, 1970; Curd, 1980; Nickles, 1980; Thagard, 1988; Josephson and Josephson, 1994; Baker, 1996; Hacking, 2001; Magnani, 2001; Psillos, 2002, 2007, 2011; Godfrey-Smith, 2003; Norton, 2003; Walton, 2004; Aliseda, 2006; Fitzhugh, 2005a, 2005b, 2006a, 2006b, 2008a; 2008b; 2008c, 2009, 2010a; Schurz, 2008). Abduction has the form:

[1]
- auxiliary theory(ies)/hypotheses, b
- theory(ies) relevant to observed effects, t (e.g. 'common ancestry')
- observed effects, e_1 (e.g. shared characters)

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- explanatory hypothesis(es), h (e.g. cladograms).

Abduction is non-deductive, as indicated by the double line separating premises (upper) from conclusion(s) (lower); deductive arguments are denoted by a single line separating premises and conclusion. Operationally, while abduction supplies hypotheses that at least initially account for observed effects, potential test evidence required to empirically evaluate the causal claims in hypotheses are predicted deductively:

[2]
- auxiliary theory(ies)/hypotheses, b
- theory(ies) relevant to the observed effects, t
- specific causal conditions presented in explanatory hypothesis via **[1]**
- proposed conditions needed to perform test

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- observed effects, e_1, originally prompting h (cf. **[1]**)
- 'predicted test evidence', i.e. effects related as closely as possible to the specific causal conditions of the hypothesis.

Induction *sensu stricto* is the subsequent act of testing hypotheses:

[3]
- auxiliary theory(ies)/hypotheses, b
- theory(ies) relevant to observed effects, t
- test conditions performed
- confirming/disconfirming evidence, e_2 (observations of 'predicted test evidence' in **[2]**, or alternative observations)

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- h is confirmed/disconfirmed.

Note that the premises in **[3]** comprise the 'test evidence'. But of this evidence, it is the observations that ensue from the act of testing (third and fourth premises), either in the form of 'predicted test evidence' inferred in **[2]** or alternative results, that stand as 'test evidence' that confirms or disconfirms, respectively, the hypothesis.

While there is the assumption that the premises used in inferences of any kind are true, only deduction can provide a

conclusion that is guaranteed true if the premises are true. In other words, the rules for valid deduction limit the conclusion to being a restatement of what is in the premises (Salmon, 1984b; Copi and Cohen, 1998). The conclusions derived from abductions and inductions are probabilistic rather than certain since the content of conclusions can extend beyond that of the premises.

From a Bayesian perspective, abduction provides the basis for the prior probability of a hypothesis, $P(h \mid e_1, b)$, and induction the posterior probability, $P(h \mid e_2, b)$. Note that 'evidence' in both **[1]** and **[3]** consists of the respective premises (Longino, 1979; Salmon, 1984b; Achinstein, 2001; Fitzhugh, 2012).[1] It is worth mentioning that while we speak of evidence as the premises in any form of inference that leads to conclusions, 'evidence' in the form of character data allowing for abductive inferences of cladograms is in sharp contrast to the 'test evidence' required to empirically evaluate those hypotheses (cf. Fitzhugh, 2006a, 2010a, 2012).

Regarding systematics, the inferences of phylogenetic hypotheses, indeed all taxa, are abductive (Fitzhugh, 2006a, 2012, 2013). Following the form in **[1]**, inferences of phylogenetic hypotheses should exhibit the following schematic structure:

[4] • ***Phylogenetic theory*:** If character $x(0)$ exists among individuals of a reproductively isolated, gonochoristic or cross-fertilising hermaphroditic population, and character $x(1)$ originates by mechanisms $a, b, c \ldots n$, and becomes fixed within the population by mechanisms $d, e, f \ldots n$ (= ancestral species hypothesis), followed by event(s) $g, h, i \ldots n$, wherein the population is divided into two or more reproductively isolated populations, then individuals to which descendant species hypotheses refer would exhibit $x(1)$.

• ***Observations (effects)*:** Individuals to which specific hypotheses *x-us* and *y-us* refer have ventrolateral margins with appendages in contrast to smooth as seen among individuals to which other species hypotheses (*a-us*, *b-us*, etc.) refer.

• ***Causal conditions (phylogenetic hypothesis* X-us*)*:** Ventrolateral margin appendages originated by some unspecified mechanism(s) within a reproductively isolated population with smooth ventrolateral margins, and the appendage condition became fixed in the population by some unspecified mechanism(s) (= ancestral species hypothesis), followed by an unspecified population-splitting event(s) that resulted in two or more reproductively isolated populations.

Note that while the formal name *X-us* would be graphically represented as a cladogram, i.e. ((*a-us*, *b-us* (*x-us*, *y-us*))), what is significant is that such a diagram implies the 'causal conditions' of character origin/fixation and population-splitting events.

The form of the 'phylogenetic theory' in **[4]** is determined by a necessary conceptual link that must exist between 'observed effects' in the form of differentially shared characters and the 'phylogenetic theory' (Fitzhugh, 2012); that link being the why-questions we implicitly or explicitly ask (Fitzhugh, 2006c, 2012) regarding those effects:

[5] 'Why do individuals to which specific hypotheses *x-us* and *y-us* refer have ventrolateral margins with appendages in contrast to smooth, as seen among individuals to which other species hypotheses (*a-us*, *b-us*, etc.) refer?'

As we are confronted with surprising or unexpected phenomena requiring explanation, in the form of differentially shared characters among organisms, what follows are the why-questions that prompt abductive inferences to phylogenetic hypotheses. The analyses by Fitzhugh (2006c, 2008b, 2012, 2013) have shown that those why-questions are located within the data matrix, where the designations of outgroups contribute to what is known as the contrastive nature of why-questions (Salmon, 1984a, 1989; Sober, 1986, 1994; Van Fraassen, 1990; Lipton, 2004; Fitzhugh, 2006a; 2006b; 2006c). This contrastive form distinguishes what is in need of explanation ('Why do individuals to which specific hypotheses *x-us* and *y-us* refer have ventrolateral margins with appendages …'), from what has been previously explained ('… in contrast to smooth, as seen among individuals to which other species hypotheses (*a-us*, *b-us*, etc.) refer?'). Why-questions seek common cause answers by way of the fact that observation statements of shared similarities carry the presupposition that those statements are true (Bromberger, 1966; Sober, 1986, 1988; Marwick, 1999; Sintonen, 2004; Schurz, 2005). Given this presupposition, explaining those similarities should involve causes that maintain as much as possible the truth of the observation statements, and that is achieved by way of a theory that ensures common causes as much as possible. Hence, the 'phylogenetic theory' in **[4]** is consistent with the presuppositions of why-questions implied in data matrices, and thus necessary. The impact of this issue on phylogenetic inference, especially regarding so-called 'likelihood' and 'Bayesian' methods, will be mentioned later (cf. 'Defeasible arguments against the requirement of total evidence').

The requirement of total evidence

It was noted in the previous section that abduction, like induction *sensu stricto*, is non-deductive, such that regardless of the truth of the premises, conclusions are only probable, as opposed to certain *qua* deduction. The consequence is that 'initial' credibility of abductive conclusions, tentative though they are, must be judged against the content of the premises. Excluding evidence that has the potential, either positively or negatively, to alter belief in, or support for a conclusion directly impinges on acceptance of that conclusion. While there are no general rules of non-deductive logic dictating the content of premises, there is the principle known as 'the requirement of

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1 The prior probability, $P(h \mid e_1, b)$, is typically shown as $P(h)$. Since the evidence in abduction is known, i.e. $P(e_1) = 1$, then $P(h \mid e_1, b) = P(h)$. As noted by Williamson (2000: 187), "… *e* itself should not be built into the background information, for that would give $P(e)$ the value 1, in which case $P(h \mid e)$ and $P(h)$ would be equal and *e* would not be evidence for anything". The negative implications for how systematists routinely refer to character data as 'supporting evidence' for cladogram topologies are significant (cf. Fitzhugh, 2012).

total evidence' that determines the degree to which rational credibility should be assigned to hypotheses (Carnap, 1950; Barker, 1957; Hempel, 1962, 1965, 1966, 2001; Salmon, 1967; 1984a, 1984b, 1989, 1998; McLaughlin, 1970; Sober, 1975; Fetzer, 1993; Fetzer and Almeder, 1993; Fitzhugh, 2006b; Kelly, 2008; Neta, 2008). Carnap (1950: 211, emphasis original) provided the first explicit description of the requirement:

"'*Requirement of total evidence*': in the application of inductive logic to a given knowledge situation, the total evidence available must be taken as basis for determining the degree of confirmation."

While the context of Carnap's characterisation is inductive, the requirement applies to all non-deductive reasoning. Failure to consider this more inclusive application has led some systematists (e.g. Wheeler, 2012: 73) to incorrectly justify the requirement via the conflation of phylogenetic inference with testing.

If the goal of scientific inquiry is the continued pursuit of causal understanding of phenomena we encounter, and evidence is that which justifies belief in the hypotheses that afford us that understanding, then deciding what evidence to consider in the derivations of beliefs will be of paramount importance. The requirement of total evidence provides the basis for choosing. Hempel (1962: 138) cogently describes the situation: "The general consideration underlying the requirement of total evidence is obviously this: If an investigator wishes to decide what credence to give to an empirical hypothesis or to what extent to rely on it in planning his actions, then rationality demands that he take into account all the relevant evidence available to him; if he were to consider only part of that evidence, he might arrive at a much more favorable, or a much less favorable, appraisal, but it would surely not be rational for him to base his decision on evidence he knew to be selectively biased."

In speaking of systematics, with the popular approaches of comparing phylogenetic hypotheses inferred from different datasets, or mapping characters on to a pre-existing set of hypotheses, i.e. cladograms, Hempel's (1966: 177, emphasis original) remarks are particularly illuminating: "When two sound inductive arguments thus conflict, which conclusion, if any, is it reasonable to accept, and perhaps act on? If the available evidence includes the premises of [two different] arguments, it is irrational to base our expectations concerning the conclusions exclusively on the premises of one or the other of the arguments; the credence given to any contemplated hypothesis should always be determined by the support it receives from the *total* evidence available at the time ... What the requirement of total evidence demands, then, is that the credence given to a hypothesis *h* in a given knowledge situation should be determined by the inductive support, or confirmation, which *h* receives from the total evidence *e* available in that situation."

In the event one is determining the plausibility of a hypothesis, whether as the product of abduction or induction, the requirement of total evidence provides a basis for assuring that plausibility is considered by way of all relevant evidence available to an investigator.[2] This is a matter of judging what premises are being used to support a particular conclusion, cf. **[1]** and **[3]**. Note that Hempel (1966) speaks of rationality when it comes to deciding theory or hypothesis acceptance. Scientific inquiry is rational to the extent we accept that theories and hypotheses are true, and that they lead to true beliefs, given available evidence. The requirement of total evidence is one of the basic tools to ensure rationality.

Since our present interest is with abduction specifically, it would be useful to look at an example of the implications of the requirement of total evidence on that type of reasoning. Consider the following abductive argument, where I attempt to explain why my lawn is wet:

[6] • When it rains, the grass gets wet
• My lawn was wet this morning

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• It must have rained last night.

The basis for the abduction would follow from the (contrastive) why-question (cf. **[5]**), 'Why is my lawn wet in contrast to being dry?' Questioning the initial plausibility of the hypothesis would entail determining if there are available premises that have been excluded or not considered. For instance, if we consider other premises (in italics), the plausibility of the conclusion in **[6]** drops substantially:

[7] • *My lawn sprinklers turn on automatically at 4 am every day*
• My lawn was wet this morning
• *The grass is dry in adjacent yards*

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• The lawn sprinklers watered the lawn last night.

Notice that the contradictory conclusions in **[6]** and **[7]** are permissible given their respective premises. The requirement of total evidence imposes no rules on how non-deductive reasoning itself should take place, but rather provides a necessary criterion of rationality for accepting the conclusions from inferences based on available evidence, i.e. the premises. If we are aware of the additional premises in **[7]**, it would be

2 It is routine in systematics that inclusion of 'all relevant/available evidence' in abduction might not be immediately practical. For instance, it is often the case that various classes of 'morphological' characters are known across a group of organisms, but other classes of characters , e.g. cilia patterns, internal anatomy, ultrastructure, nucleotide sequences, etc., are sporadically available. Inclusion of these latter data can necessitate an abundance of 'unknown' (i.e. '?') codings, resulting in explanations (transformation series) that are largely uninformative within the scope of organisms considered. It might be more effective to delay explaining these latter observations until more inclusive coverage is attained. This is *not* to suggest that some classes of characters should be explained separately from others. The requirement of total evidence stipulates an ideal for inclusion of evidence. The goal with regard to abduction is to get as close as possible to that ideal within the limits of epistemic feasibility. Alternatively, if one wishes, for instance, to explain sequence data for a limited group of organisms for which other data are readily available, e.g. 'morphological' characters, the requirement of total evidence decisively mandates that these latter data be explained within the same abductive inference as those sequence data.

less rational to accept the conclusion in **[6]**. We recognise that considering these latter premises makes the initial conclusion less credible relative to the causal account that relies on the more inclusive available evidence that can affect plausibility:

[8]
- There are no records of rainfall last night
- My lawn sprinklers turn on automatically at 4 am every day
- My lawn was wet this morning
- The grass is dry in adjacent yards

- The lawn sprinklers watered the lawn last night.

An analogous situation will be examined in the next section for phylogenetic inferences.

The requirement of total evidence and systematics: epistemic issues

With the basics of abductive reasoning and the requirement of total evidence presented in the previous sections, we can identify implications for two common approaches in systematics: comparing cladograms inferred from different datasets, and mapping characters on cladograms inferred from other data.

Comparing cladograms

The practice of inferring phylogenetic hypotheses from separate sets of why-questions *qua* partitioned datasets, with subsequent comparisons of topologies, also known as 'taxonomic congruence,' has a lengthy history (e.g. Mickevich, 1978). The most popular approach at present is to compare cladogram topologies inferred from 'morphological' and sequence data, respectively, or between 'morphological' and different sets of sequence data.

Using the schematic example in fig. 1A, the most basic problem with cladogram comparison can be identified. Separate abductive inferences (cf. **[1]**, **[4]**) accounting for observations in datasets α and β are implied by the respective topologies, (*a-us* (*b-us* (*c-us*, *d-us*))) and ((*a-us*, *b-us*) (*c-us*, *d-us*)). The letters on each cladogram 'node' indicate hypotheses of population-splitting events necessary to explain the data in conjunction with hypotheses of character origin and fixation ['transformation series,' i.e. $n(0 \rightarrow 1)$]. Whether or not the theories used (cf. ***Phylogenetic theory*** in **[4]**) in the two inferences are the same will not matter at the moment. Note that the respective conclusions are contradictory in that they hypothesise the past existence of different sets of causal conditions. Strictly speaking, however, the causal events of character origin/fixation are assumed to be independent of one another. This assumption is required for the fact that we ask separate why-questions (cf. **[5]**) regarding different characters, and operate under the view that those observations need to be explained by separate or independent causal events of character origin and fixation among members of reproductively isolated ancestral populations (Fitzhugh, 2006a, 2006c, 2008c, 2012). But when we take population-splitting events into account, problems with cladogram comparison become apparent.

Consider population-splitting event B in fig. 1A. In conjunction with the hypotheses of character origin/fixation of characters 2(1), 3(1), and 4(1) among members of an ancestral population, splitting event B also explains the presence of those characters among individuals to which specific hypotheses *b-us*, *c-us* and *d-us* also refer. Next consider population-splitting events E and F in the other cladogram. Hypothesis E partially explains character 7(1) among individuals to which *a-us* and *b-us* refer, while hypothesis F accounts in part for character 8(1) among individuals to which *c-us* and *d-us* refer. What is immediately apparent is that hypothesis B contradicts hypotheses D, E and F, and vice versa. The plausibilities of the individual hypotheses are compromised because they account for respective observations with conflicting causal events of the same class. Hypothesis B could not be rationally accepted relative to hypotheses D–F. Contradictory sets of population-splitting events are decisive for acknowledging that the composite hypotheses represented by cladograms impinge on our ability to rationally explain all available, relevant observations. It is also the case that the separate hypotheses of character origin/fixation implied by the two cladograms call into question the credibility of those classes of hypotheses. For instance, explaining characters 2(1) through 5(1) influence rational acceptance of hypotheses for characters 7(1) and 8(1), and vice versa. The solution is to infer causal accounts for both sets of characters within the same inference (fig. 1B). Indeed, this is a constraint immediately apparent from the perspective mentioned earlier, that why-questions (cf. **[5]**) determine the conceptual link between observation statements and the theory that must be uniformly applied to those statements (cf. **[4]**).

Related to the issue of contradictory population-splitting events just described (fig. 1A), there is an additional problem that has received insufficient attention. It is not uncommon, especially with the separate inferences of phylogenetic hypotheses for 'morphological' and sequence data, that different theories are employed. As the only solution to rationally decide between contradictory hypotheses of population-splitting events is to apply the requirement of total evidence (fig. 1B), this also entails that the same theory(ies) be used for all available observations being explained. The matter of what theory(ies) to use in the inferences of phylogenetic hypotheses lies beyond the scope of this paper.[3] Regardless, there are substantial epistemic difficulties associated with most phylogenetics-related theories due to the fact that relations between observations, why-questions, and abductive inferences required to answer those questions have been largely overlooked (Fitzhugh, 2006a, 2006b, 2006c, 2008c, 2012, 2013). In lieu of combining data, the only alternative is to segregate out those why-questions that would not require phylogenetic hypotheses as answers, but rather one of the other classes of hypotheses, e.g. intraspecific or specific. Such attention to detail is, however, rarely considered.

An obvious consequence of the analysis presented thus far is that phrases of the form 'Morphological and molecular

3 Albeit the ***Phylogenetic Theory*** in **[4]** is sufficient for the why-questions asked in systematics (cf. **[5]**) (Fitzhugh, 2012). In terms of presenting causal events accounting for shared characters, cladograms are remarkably vague in their details.

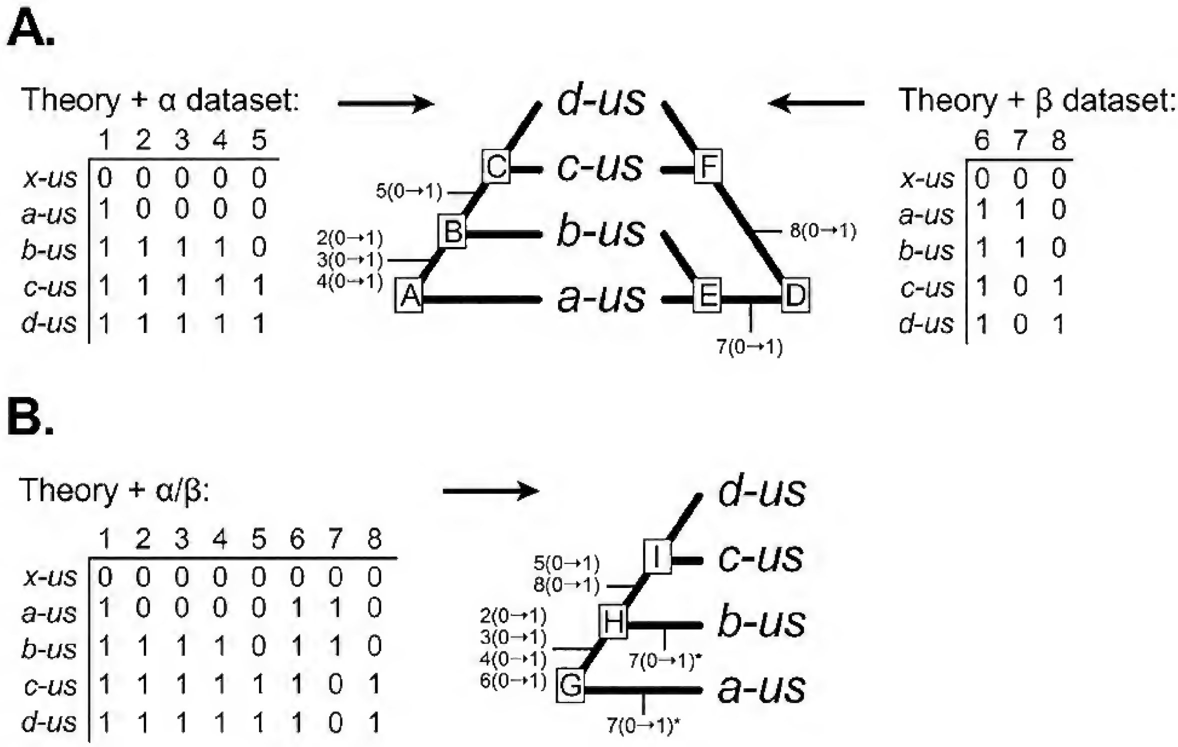

Figure 1. Example of the error of cladogram comparisons. A, phylogenetic hypotheses inferred from separate sets of premises. Letters on cladogram 'nodes' indicate population-splitting events relevant to the various hypotheses of character origin/fixation within ancestral populations. The requirement of total evidence precludes such a comparison of cladogram topologies because explanations of characters 1(1)–5(1) by population-splitting events A–C (left cladogram) contradict explanations of 6(1)–8(1) by population-splitting events D–F. See text for further discussion. B, explaining observations in accordance with the requirement of total evidence, correcting the problem in 'A'.

phylogenies for group *X* disagree (or agree)' are epistemically meaningless. There can be no disagreement/agreement due to the fact that the objective of phylogenetic inference is not to obtain 'trees'. Cladograms, as branching structures, are only as scientifically informative as the hypotheses of past causal events that can be discerned from such diagrams, as answers to why-questions. To speak of 'disagreement' among 'phylogenies' or cladograms as branching structures is to commit the fallacy of reification; treating cladograms as the tangible objects of interest rather than the actual hypotheses implied by those diagrams. The only disagreements that can be referred to among cladograms inferred from different sets of data are hypotheses of character origin/fixation within ancestral populations and subsequent population-splitting events (cf. fig. 1A); both being the result of failing to follow the requirement of total evidence (*pace* fig. 1B).

Character mapping

The popular alternative to separate inferences of phylogenetic hypotheses for partitioned data is the use of cladogram topologies based on one set of data as the 'framework' for determining phylogenetic hypotheses for other data not involved in the inference of a cladogram (i.e. not present in the premises; cf. **[1]**, e_1). As with cladogram comparisons discussed earlier, the issue here will be to show that decisions regarding the plausibility of phylogenetic hypotheses are compromised because mapping involves inferential processes separate from inferences of the cladograms-as-phylogenetic hypotheses upon which characters are mapped.

Fig. 2A presents an abductive inference for a set of observed effects—dataset α—where the cladogram implies at a minimum the two classes of causal events of character origin/fixation and subsequent population splitting. Also represented are the separately inferred species hypotheses, *a-us* through *d-us*. Using this cladogram topology, additional observations—dataset β—are then 'mapped' on to 'branches' of the cladogram (fig. 2B), generally in a presumptive effort to 'optimise' placements of characters to minimise *ad hoc* hypotheses of homoplasy.

Character mapping fails as a scientifically viable approach because it is in essence a variant of cladogram comparison. As discussed in the previous section, the phylogenetic hypotheses

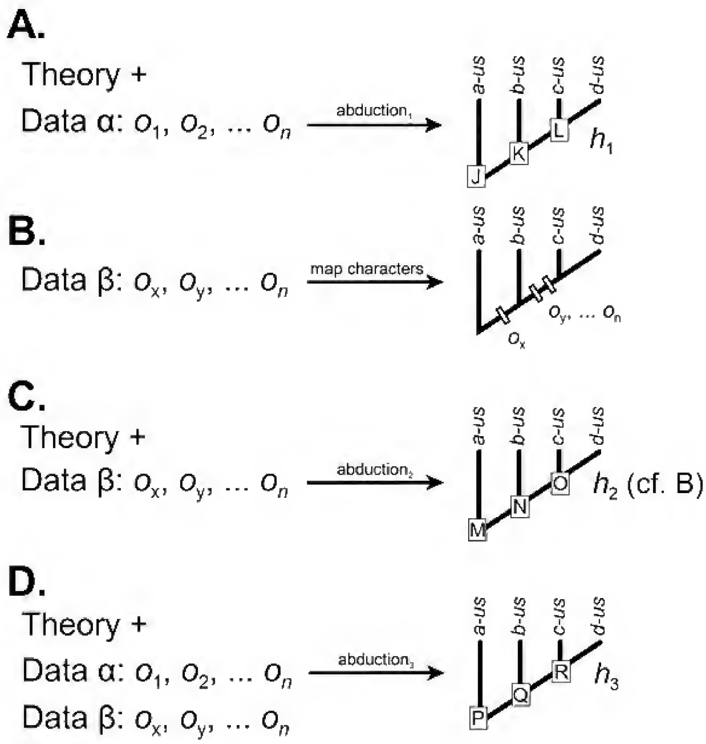

Figure 2. Example of the error of character mapping. A, phylogenetic hypotheses are inferred for a set of characters. Numbers on cladogram 'nodes' indicate population-splitting events relevant to the various hypotheses of character origin/fixation within ancestral populations (not shown; cf. fig. 1). B, a different set of characters are 'mapped' onto the branches of the cladogram in 'A'. C, the 'mapped' characters in 'B' actually refer to phylogenetic hypotheses inferred separately from the hypotheses implied by the cladogram in 'A' and 'B'. D, explaining observations in accordance with the requirement of total evidence, correcting the problem in 'B' and 'C'. See text for further discussion.

(fig. 2A) inferred using dataset α are only relevant to those characters, as explanatory accounts. While mapping (fig. 2B) gives the appearance of conjoining additional observations to these hypotheses to produce a more inclusive set of explanations, this is not the case. Regardless of what characters are mapped on to a previously inferred cladogram, the transformation series for the mapped characters do in fact represent consequences of inferential acts, albeit quite vague, that are wholly separate from the initial inference (fig. 2C). As composite hypotheses, cladograms h_1 and h_2 in fig. 2A and 2B/C, respectively, refer to different sets of explanatory accounts. The fact that the cladograms have the same topologies has no epistemic standing. Topologies of branching diagrams are immaterial. What matters are the causal events conveyed by those diagrams as answers to why-questions. The population-splitting events in h_1 (fig. 2A) only pertain to explanations of α-type characters, while events in h_2 (fig. 2B/C) only relate to β-type characters, yet both sets of hypotheses refer to classes of events that directly impinge on the credibility of those hypotheses. Per the requirement of total evidence, the only solution is that both sets of characters must be explained via the same abductive inference (fig. 2D).

Defeasible arguments against the requirement of total evidence

Cladogram comparisons and character mapping have become accepted practices in biological systematics on the basis of two common arguments endorsing the partitioning of character data: (i) sets of characters are so different in quality, or subject to radically dissimilar causal processes, as to require separate treatment, and (ii) classes of data with inordinately disparate representation will result in the 'signal' or 'noise' from the larger class 'overwhelming' what can be offered by the smaller class. Most often the perceived need for partitioning falls along the arbitrary lines of 'morphology' and nucleotide or amino acid sequences. Partitioning has never been defended on the basis of presenting a valid alternative to the requirement of total evidence that indicates the requirement is defective and at the same time establishes that partitioning promotes a more rational evaluation of hypothesis credibility in relation to abductive reasoning (cf. Fitzhugh, 2006b, 2008c). In this section, arguments (1) and (2) are shown to be invalid.

'Characters cannot be combined'

Claiming that a particular class of data, e.g. nucleotide sequences, is fundamentally different from another class, e.g. 'morphology,' such that phylogenetic hypotheses explaining the former must be inferred separately from phylogenetic hypotheses explaining the latter suffers from several basic oversights. Recall that aligning systematics with all fields of science requires acknowledging that the objective is to acquire causal understanding of differentially shared characters among organisms. This goal, via why-questions (cf. **[5]**) leading to abductive inferences (cf. **[4]**), provides the conceptual link between our observation statements of the properties of organisms and the explanatory hypotheses referred to as taxa (Fitzhugh, 2005b, 2008b, 2009, 2010b, 2012, 2013; Nogueira et al., 2010, 2013). There are two aspects of this conceptual link that have been almost uniformly overlooked in systematics, especially with regard to developments of algorithms for phylogenetic inference: the why-questions related to our observations (cf. **[5]**) and the nature of abductive reasoning required to provide at least initial answers to those questions (cf. **[1]**, **[4]**). Indeed, while principles of phylogenetic inference have developed around notions like parsimony, 'likelihood,' and 'Bayesianism,'[4] the latter two have no relevance to abduction, and parsimony is only worthy of consideration in the context of the why-questions to which abduction is directed (Sober, 1975; Fitzhugh, 2006a, 2006b, 2012). All in all, what stands as the basis for phylogenetic inference is correctly applying abduction to why-questions, not deciding whether to use [*sic*] parsimony, 'likelihood,' or 'Bayesianism.'

What precludes data partitioning on the basis that classes of data are either qualitatively different or the products of substantively different causal processes is that the why-questions invariably have the form shown in **[5]**. The very nature of observation statements of shared similarities determines that why-questions seek common cause answers (cf. 'Reasoning and the requirement of total evidence', above)—a perspective that is at odds with 'likelihood' and 'Bayesian' methods in systematics (Fitzhugh, 2006a, 2012). The standard argument for 'likelihood' and 'Bayesian' phylogenetic inferences is that they take into consideration rates of sequence evolution (Felsenstein, 2004; Schmidt and von Haeseler, 2009; Ronquist et al., 2009). But once one invokes rates, this must place *a priori* constraints on our observation statements, rather than introducing rates within the abductive framework for explaining those observations relative to other observations by way of phylogenetic hypotheses. This is a direct consequence of basic logic and rationality: the assumption that premises are true propositions (Williamson, 2000). For observation statements of shared similarities to have the status of evidence/premises in abduction (e.g. **[4]**: ***Observations (effects)***), those statements must be regarded as true. The conjunction of a theory of substitution rates and shared similarities is a contradiction. Rates of sequence evolution must be considered at the point one proceeds from perceptions to observation statements. For instance, rather than accepting that individuals to which species hypotheses *x-us*, *y-us* and *z-us* refer have nucleotide A at position 234, in contrast to T, as observed among individuals to which species hypotheses *a-us*, *b-us* and *c-us* refer, a theory of substitution rates must first be used to determine which nucleotides are in fact A while others are A'. In other words, accepting a theory of substitution rates requires that one's perceptions of A first be subjected to an initial abductive inference distinguishing some A's as shared similarities that are distinct from A''s (other shared similarities). Upon making this distinction, the subsequent why-question would have the form, "Why do individuals to which species hypothesis *x-us* refers have an A at position 234, whereas individuals to which species hypotheses *y-us* and *z-us* refer have A' (in contrast to T, as observed among individuals to which species hypotheses *a-us*, *b-us* and *c-us* refer)?" The form of the why-question is a necessary consequence of applying the theory of substitution rates at the proper epistemic juncture, i.e. prior to the abductive inference of phylogenetic hypotheses, **[4]**.[5] The subsequent abductive inference directed at all relevant shared similarities would again seek common cause answers in the form of phylogenetic hypotheses.

With the correct utilisation of why-questions that require phylogenetic hypotheses as answers, there are no differences between characters that could warrant the partitioning of data that leads to cladogram comparison or character mapping. Similarly, attempts to develop methodological criteria to determine the extent to which data should be combined, such as the incongruence length difference test (Farris et al., 1995; Barker and Lutzoni, 2002), are nullified due to the fact that they operate under the incorrect assumption that cladograms can be empirically compared for the purpose of deciding whether or not the respective explanations of partitioned data should be discarded in lieu of being explained *en masse*.

4 These terms are placed in quotes because their application to abductive reasoning is erroneous (Fitzhugh, 2012). The likelihood principle refers to the probability of observing *test evidence* for a particular hypothesis, $P(e \mid h)$ (Hacking, 1965; Howson and Urbach, 1993; Lipton, 2008), while Bayesianism addresses changes in belief in hypotheses, as posterior probabilities $P(h \mid e)$, subsequent to the 'introduction of test evidence' (Salmon, 1967; Howson and Urbach, 1993; Hacking, 2001). The methods known as 'maximum likelihood' and 'Bayesianism' in systematics incorrectly conflate the abductive inferences of hypotheses with the testing of those hypotheses—a long-standing view created by equating abductive evidence, i.e. the premises in **[1]** and **[4]**, with test evidence (cf. **[2]**, **[3]**). This mistake has been extended to include the concept of statistical consistency (Felsenstein, 1981, 2004), where preferred methods should 'converge' on true [*sic*] hypotheses with the addition of more and more 'test' evidence (*not* abductive evidence). As noted by Fitzhugh (2012, see also references therein), consistency is a perspective that is meaningless in the context of abduction.

5 I doubt any systematist would find this manoeuvre practical, much less readily operational. But the only alternative is to maintain the integrity of observation statements of shared similarities in both why-questions and abductive inferences (cf. **[5]**, **[4]**, respectively). As with any field of science, calling into question whether or not shared similarities should be explained by way of some hypothesis of common cause is something considered during the process of empirical hypothesis testing, not the inferences of those hypotheses. This is yet one more reason why 'likelihood' and 'Bayesian' approaches to abductive reasoning are misguided.

'One set of data will overwhelm other data'

The intuitive appeal of the idea that the large number of nucleotides or amino acids comprising sequence data can have negative effects on the 'signal' offered by 'morphological' characters derives from two misconceptions. First, it is senseless to regard characters as either 'signal' or 'noise.' To invoke this distinction introduces the incorrect presumption that one has already explained observations prior to the abductive inferences of phylogenetic hypotheses, or is relying on specious 'support' measures like the bootstrap or Bremer index (Fitzhugh, 2006a, 2012) subsequent to inferring explanations. As the intent of phylogenetic inference is to provide answers to specifiable why-questions regarding our observation statements, there are no concepts of 'signal' and 'noise' that are applicable. Second, presuming that explaining one set of characters negatively impinges on explanations of other sets of characters requires introducing some sort of extra-evidential justification for partitioning, of which there is none. Characters considered in abductive inferences to phylogenetic hypotheses are equivalent from the perspective that they require the same explanatory structure. That equivalence is determined by the fact that the why-questions being asked (cf. **[5]**), and which are implied by a data matrix (Fitzhugh, 2006c), invoke a theory of common ancestry (cf. **[4]**, ***Phylogenetic theory***) applicable to all the observations. Rather than introducing *ad hoc* maneuvers to ensure obtaining unwarranted, preordained results, answers to why-questions need to be evaluated through the standard approach of seeking test evidence that either confirms hypotheses or points to alternatives.

Conclusions

Rationality is a fundamental feature of scientific inquiry, for it enables making empirical choices between competing hypotheses or theories. In the context of abductive reasoning, being the source of hypotheses throughout biological systematics, objectively determining initial degrees of belief between hypotheses is a matter of considering the content of premises (cf. **[1]**, **[4]**, **[6]**–**[8]**). The requirement of total evidence ensures that the basis for initially accepting one hypothesis over another, i.e. $P(h_1 \mid e_1, e_2, \ldots e_n) > P(h_2 \mid e_1)$, is a rational decision. That initial acceptance is not the same as acceptance subsequent to subjecting hypotheses to empirical testing (cf. **[2]**, **[3]**), in which case the requirement of total evidence would also apply when taking into account test evidence. Regardless of properly adhering to the requirement of total evidence, the hypotheses implied by cladograms are profoundly meager causal constructs, lacking in the details needed to even consider them worthy of testing (Fitzhugh, 2012). But, this inherent limitation does not justify the tradition of uncritical thinking that has developed within, and has become a mainstay of biological systematics.

The lack of proper consideration of the requirement of total evidence within systematics has probably been mainly due to outright disagreement with the principle and/or not fully understanding it, coupled with the historical failure to embrace abductive reasoning, and perhaps no awareness regarding the importance of rationality in science. Overlooking these factors figures prominently in, for instance, Felsenstein's (2004: 536) mistaken view that a 'total evidence debate' exists in systematics. What might be perceived as a debate is actually the conjunction of multiple misunderstandings of reasoning. No valid dispute exists on the subject within the scope of logic (Hempel, 1965; Kelly, 2008; Neta, 2008) that could warrant the perception that the requirement can be bypassed in systematics. Unless systematics is successful at devising its own unique protocols for ensuring rational reasoning—which has not been the case—there is no denying the import of the requirement of total evidence. It is an ironic twist that scientists are quick to criticise such pursuits as creationism/intelligent design because they fail at leading to scientifically acceptable conclusions. Given the choice between the well-tested theory of natural selection and an untested theory of a non-natural designer, reliance on the latter is acknowledged as offering less rational understanding than the former. Yet, we see cladogram comparisons and character mapping deemed acceptable, even though they too violate the same basic tenet of rationality. The success of scientific inquiry stands on consistently recognising the essential necessary elements for rational reasoning. Systematics cannot afford to depart from those standards by ignoring the requirement of total evidence.

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A new species of *Exogone* (Syllidae: Exogoninae) from off the state of São Paulo (south-east Brazil)

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Abstract Fukuda, M.V. and Nogueira, J.M.M. 2014. A new species of *Exogone* (Syllidae: Exogoninae) from off the State of São Paulo (south-east Brazil). *Memoirs of Museum Victoria* 71: 79–84.

We describe a new species of *Exogone* Örsted, 1845 (Syllidae: Exogoninae) found in dense populations in some areas off the State of São Paulo (south-east Brazil). *Exogone cebimar* sp. nov. has an enlarged median antenna, dorsal cirri present on all chaetigers, a triangular process on each of the shafts of spiniger-like chaetae of segments 1 and 2, and a short proventricle, extending for two segments only. This new species is one of the subjects of ongoing studies dealing with the characterisation of brooding methods found in the subfamily Exogoninae.

Keywords Araçá Bay, polychaete, intertidal, rocky shore, CEBIMar

Introduction

Despite records existing for around 140 species, the syllid fauna along the Brazilian coast is still considered largely unknown, since most of the records come from material collected from shallow waters off the south-eastern region of the country, and mostly from soft bottoms. The northern coast of the State of São Paulo, south-eastern (SE) Brazil, is one of the best-studied regions for the polychaete fauna of the intertidal zone, but even in this area it is not rare to find new occurrences and species new to science in taxonomic studies.

During studies focused on the polychaete fauna occurring off the State of São Paulo and, in particular, a recent research into the diversity and reproductive features of the Syllidae Grube, 1850, a new species of *Exogone* Örsted, 1845 was found.

This new species is abundant in the studied area, to the extent that it has been chosen as one of the target species for an ongoing research into the reproduction of Syllidae, being representative of the ventral brooding of eggs and embryos method found in the subfamily. Characterisation of this reproductive process will be presented in subsequent papers.

Materials and methods

The material analysed came from three projects focused on the biota occurring off the State of São Paulo. The first, 'Biodiversity of intertidal polychaetes on rocky shores off the State of São Paulo' ('BioPol') sampled a range of beaches comprehending most of the shoreline of São Paulo. The other two projects, 'Taxonomic study of the Syllidae (Annelida, Polychaeta) in the Araçá Bay and analysis of the incubation modes in the Exogoninae' and 'Biodiversity and functioning of a subtropical coastal ecosystem: a contribution to integrated management' ('BIOTA – Araçá')', are ongoing studies conducted on the Araçá Bay (São Sebastião, São Paulo). This bay is particularly important because it is very rich in terms of biodiversity, but it is threatened by expansion plans for the neighbouring Port of São Sebastião.

In all projects, collections were made on rocky shores from the intertidal zone at neap tides, mostly by scraping different biological substrates (sponges, ascidians, algae, etc.) from the rocks. In the laboratory, polychaetes were sorted under a stereomicroscope, relaxed in a menthol solution, fixed in 4% formaldehyde and, a few weeks later, rinsed in fresh water and preserved in 70% ethanol.

Identifications were based exclusively on morphological characters. Illustrations were done with the aid of a drawing tube attached to an Olympus® BX-51 microscope. Length of specimens was measured from the tip of the palps to the tip of the pygidium, excluding anal cirri; width was measured at proventricle level, excluding parapodia. Blade lengths for

compound chaetae are provided in dorso-ventral sequence. For scanning electron microscope (SEM) observation, specimens were dehydrated in a series of ethanol solutions with progressively increasing concentrations up to 100%, critical-point dried, covered with a 10–20 nm layer of gold, and then observed under the SEM at the Laboratório de Microscopia Eletrônica, Instituto de Biociências, Universidade de São Paulo.

Abbreviations

Abbreviations for museum names are as follows:

AM — The Australian Museum, Sydney, Australia

MNCN — Museo Nacional de Ciencias Naturales, Madrid, Spain

MZUSP — Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil

ZUEC — Museu de Zoologia da Universidade Estadual de Campinas, Campinas, Brazil

ZMH — Zoologisches Museum Hamburg, Hamburg, Germany

Systematics

Family **Syllidae** Grube, 1850

Subfamily **Exogoninae** Langerhans, 1879

Genus ***Exogone*** Örsted, 1845

Type species. Exogone naidina Örsted, 1845.

Diagnosis. Relatively small, thin and slender bodies. Palps well developed, completely fused or with terminal notch. Prostomium ovate, with 2 pairs of eyes in trapezoidal arrangement and, sometimes, 1 pair of anterior eyespots; 3 smooth antennae, all short and ovate, or at least median antenna elongate, digitiform. Peristomium with 1 pair of peristomial cirri. Dorsal cirri present on all chaetigers or absent on chaetiger 2. Peristomial, dorsal and ventral cirri short, papilliform to ovate. Compound chaetae with subdistally inflated and spinulated shafts; in some species, shafts with conspicuous subdistal triangular enlargement ('triangular process') on spiniger-like chaetae of a few anterior parapodia. Blades of falcigers usually spinulated, bidentate, distal tooth smaller than subdistal one; dorsalmost compound chaetae frequently with long and slender spiniger-like blades, with short spinulation. In some species, compound chaetae secondarily simple by fusion of shaft and blade, or by loss of blade. Dorsal simple chaetae present from anterior body, usually sigmoid, progressively stouter posteriorwards; dorsal simple chaetae bayonet-like in some species. Ventral simple chaetae usually present only on posteriormost chaetigers, bidentate, distal tooth smaller than subdistal one. Aciculae distally inflated, apparently hollow, with slightly bent tip. Pygidium with one pair of anal cirri, usually longer than dorsal cirri along body (San Martín, 2005).

Exogone cebimar sp. nov.

Zoobank LSID. http://zoobank.org/urn:lsid:zoobank.org:act:0D7F6ADA-B2A7-469D-8594-E4D6240028C9

Figures 1–2, table 1.

Material examined. Project 'BIOPOL'. São Sebastião – Praia do Araçá (23°48'54"S 45°24'24"W): 1 spec., 17 Apr 2003; 15 specs, 15 Jul 2003; 18 specs, 25 Sep 2003; Praia Preta (23°49'16"S 45°24'35"W): 1 spec., 18 Apr 2003; 8 specs, 18 Jul 2003. São Vicente – Ilha Porchat (23°58'39"S 46°22'08"W): 1 spec., 15 Jun 2003; Praia das Vacas (23°58'55"S 46°22'48"W): 1 spec., 16 May 2003.

Project 'BIOTA-Araçá'. São Sebastião – Praia do Araçá (23°48'54"S 45°24'24"W): 2 specs, 18 May 2011; 16 specs, 25 Sep 2011; 6 specs, 21 Nov 2011; 6 specs, 22 Feb 2012; 72 specs, 7 May 2012; 19 specs, 30 Sep 2012; 75 specs (holotype, MZUSP1966; paratype 1, MZUSP 1967; paratype 2, ZUEC-Pol 14101; paratypes, MZUSP 1968), 1 Oct 2012; 2 specs, 2 Oct 2012.

Type material. Data of the holotype and two selected paratypes are provided in table 1, all specimens collected by Project 'BIOTA-Araçá', 1 Oct 2012.

Comparative material examined. Exogone lourei Berkeley and Berkeley, 1938. Pacific Ocean, Australia – Western Australia, Goss Passage, Beacon Island (28°25'30"S 113°47'E): 12 specs (AM W26992), coll. P. Hutchings, 22 May 1994, det. G. San Martín, 2001. Atlantic Ocean, Cuba – Canarreos Archipelago, Isla de la Juventud, Punta del Francés: 3 specs. (MNCN 16.01/630), leg. & det. G. San Martín. Cape Verde – Sal Island, Joaquim Petinha: 3 specs. (MNCN 16.01/6909), coll. & det. G. San Martín, 8 Aug 1985.

Exogone multisetosa Friedrich, 1956. Pacific Ocean, Peru – Lima: 3 specs (ZMH P-15371, holotype; P-15372, paratypes), coll. Remane, 22 Jun 1952, det. Friedrich, 1956.

Description. Body usually orange in colour in live specimens, thin and elongate, holotype largest specimen analysed, 7.78 mm long, 0.23 mm wide, with 46 segments (table 1). Palps ovate, elongate, almost totally fused, with distal notch (figs 1A; 2A–B, D). Prostomium ovate, shorter than palps, with 2 pairs of eyes in trapezoidal arrangement; anterior eyespots absent; median antenna inserted slightly anterior to anterior pair of eyes, elongate, almost reaching tip of palps, subdistally inflated, distally tapering; lateral antennae inserted close to median antenna but slightly anteriorly, ovate, short, almost 1/3 length of median antenna (figs 1A; 2A–B, D). Peristomium slightly shorter than subsequent segments; peristomial cirri ovate, short, smaller than lateral antennae; nuchal organs as 1 pair of dorsolateral short ciliated slits, close to border between prostomium and peristomium (fig. 2E). Dorsal cirri present on all chaetigers, ovate, slightly larger than peristomial cirri but smaller than lateral antennae on anterior body, with slight increase in size and more tapered distally, ovate to pyriform, towards posterior body; ventral cirri similar to dorsal cirri of corresponding parapodium but smaller, ~1/2–2/3 length of corresponding parapodial lobe (fig. 2B–D). Parapodial lobes conical (figs 1A; 2A–D). Shafts of compound chaetae subdistally spinulated, spines arranged in thin rows on midbody chaetae (fig. 1D). Anterior and midbody parapodia with 1, sometimes 2 spiniger-like chaetae each, posterior body parapodia with single spiniger-like chaetae each; spiniger-like chaetae of chaetigers 1 and 2 with subdistal short triangular

Table 1. Morphological variation among selected specimens of the type series of *E. cebimar* sp. nov. All specimens were collected at Praia do Araçá (23°48'54"S 45°24'24"W) on the rocky shore, intertidal zone, 1 Oct 2012.

Exogone cebimar sp. nov.	**Holotype** MZUSP 1966	**Paratype 1** MZUSP 1967	**Paratype 2** ZUEC-Pol 14101
Number of chaetigers	46	42	43
Total length x width at proventricle (mm)	7.78 x 0.23	6.62 x ~0.20	7.00 x ~0.17
Length of blades of spiniger-like chaetae (μm)/number of spiniger-like chaetae per parapodium			
Anterior body	50–37/1	42–31/1	42–36/1
Midbody	45–32/1-2	42–35/1	45–35/1–2
Posterior body	~22/1	22–18/1	22–18/1
Length of blades of falcigers (μm)/number of falcigers per parapodium			
Anterior body	10–7.5/5–6	10–7.5/5–7	10–7.5/5–7
Midbody	~7.5/3–4	~7.5/3–4	~7.5/3–4
Posterior body	~7.5/2–3	7.5–5/2–3	7.5–5/2–3
Length of pharynx (chaetigers)	4	5	4
Length of proventricle (chaetigers); number of muscle cell rows	2; ~20	2; ~20	2; ~21

process on shafts (figs 1B–C; 2F–G); blades spinulated, inconspicuously bifid, 50–31 μm long on anterior body, 45–32 μm on midbody, 22–18 μm on posterior body (table 1). Anterior parapodia with 5–7 falcigers each, midbody with 3–4, posterior parapodia with 2–3 falcigers each; blades of falcigers bidentate and spinulated (figs 1D–E; 2G); slight dorsoventral gradation in length, blades 10–7.5 μm long on anterior body, ~7.5 μm on midbody, 7.5–5 μm long on posterior body (table 1). Dorsal simple chaetae present from anterior body, sigmoid, subdistally spinulated, with thin tip, progressively stouter posteriorwards (figs 1F–G; 2H); ventral simple chaetae only present on posteriormost chaetigers, sigmoid, bidentate, tips resembling those of falciger blades, about as thick as dorsal simple chaeta of corresponding parapodium (figs 1H; 2I). Anterior parapodia with up to 3 aciculae each, 2 of which are distally inflated, apparently hollow, one straight, other distally oblique, remaining acicula straight, distally tapering (fig. 1I); number of aciculae per parapodium decreasing towards posterior body, posterior parapodia with single acicula each, stouter than on anterior body parapodia, distally inflated, with slightly oblique tip (fig. 1J). Pygidium with elongate anal cirri, slightly longer than median antenna (fig. 2C). Pharynx through 4–5 chaetigers, anterior margin surrounded by ~10 soft papillae (fig. 2D), inner margin of pharynx chitinised; large conical tooth close to opening; proventricle through ~2 chaetigers, with ~20 muscle cell rows (fig. 1A; table 1).

Remarks. *Exogone cebimar* sp. nov. differs from all other species in the genus by the following combination of characters: median antenna longer than lateral ones, almost reaching tip of palps, subdistally inflated, distally tapering; dorsal cirri present on chaetiger 2; shafts of spiniger-like chaetae from chaetigers 1 and 2 subdistally with short triangular process; and proventricle short, through ~2 chaetigers.

Exogone cebimar sp. nov. belongs to a group of species with a triangular process on the shaft of each spiniger-like chaeta of some anterior body chaetigers. This group also includes *E. arenosa* Perkins, 1981, *E. lourei* Berkeley and Berkeley, 1938, *E. multisetosa* Friedrich, 1956, *E. pseudolourei* San Martín, 1991, *E. rostrata* Naville, 1933, and *E. uniformis* Hartman, 1961. Of all these species, however, only *E. lourei* has that process occurring on both chaetigers 1 and 2, as in *E. cebimar* sp. nov., all other species having it on a single chaetiger, either 1 or 2.

Exogone lourei, however, is a larger species, differing from *E. cebimar* sp. nov. in having a longer proventricle, extending for 4–5 chaetigers, instead of ~2 chaetigers, as in *E. cebimar* sp. nov. Furthermore, the triangular processes on the shafts of spiniger-like chaetae of *E. cebimar* sp. nov. are different from those of *E. lourei* and all other species in this group, as in all other species it is a larger structure, frequently larger than the width of the distal part of the shaft, and it is inserted at 90° to the shaft, whereas in *E. cebimar* sp. nov., the triangular processes are smaller, roughly pointed triangles coming out of the shaft.

The chitinised lining of the pharynx in this species frequently forms small fractures in the opening, probably due to abrasion while feeding. In some cases, these fractures resemble small teeth, as found in species that have a trepan, however, in dissected specimens of *Exogone cebimar* sp. nov., we did not see any sign of teeth other than the central pharyngeal tooth.

Etymology. The species is named after the 'Centro de Biologia Marinha da Universidade de São Paulo' ('CEBIMar – USP'), whose facilities are used by many researchers working on different marine-related fields. The existence of this institution on the northern coast of the State of São Paulo can be considered one of the main reasons for it being one of the best-studied regions of the Brazilian coast.

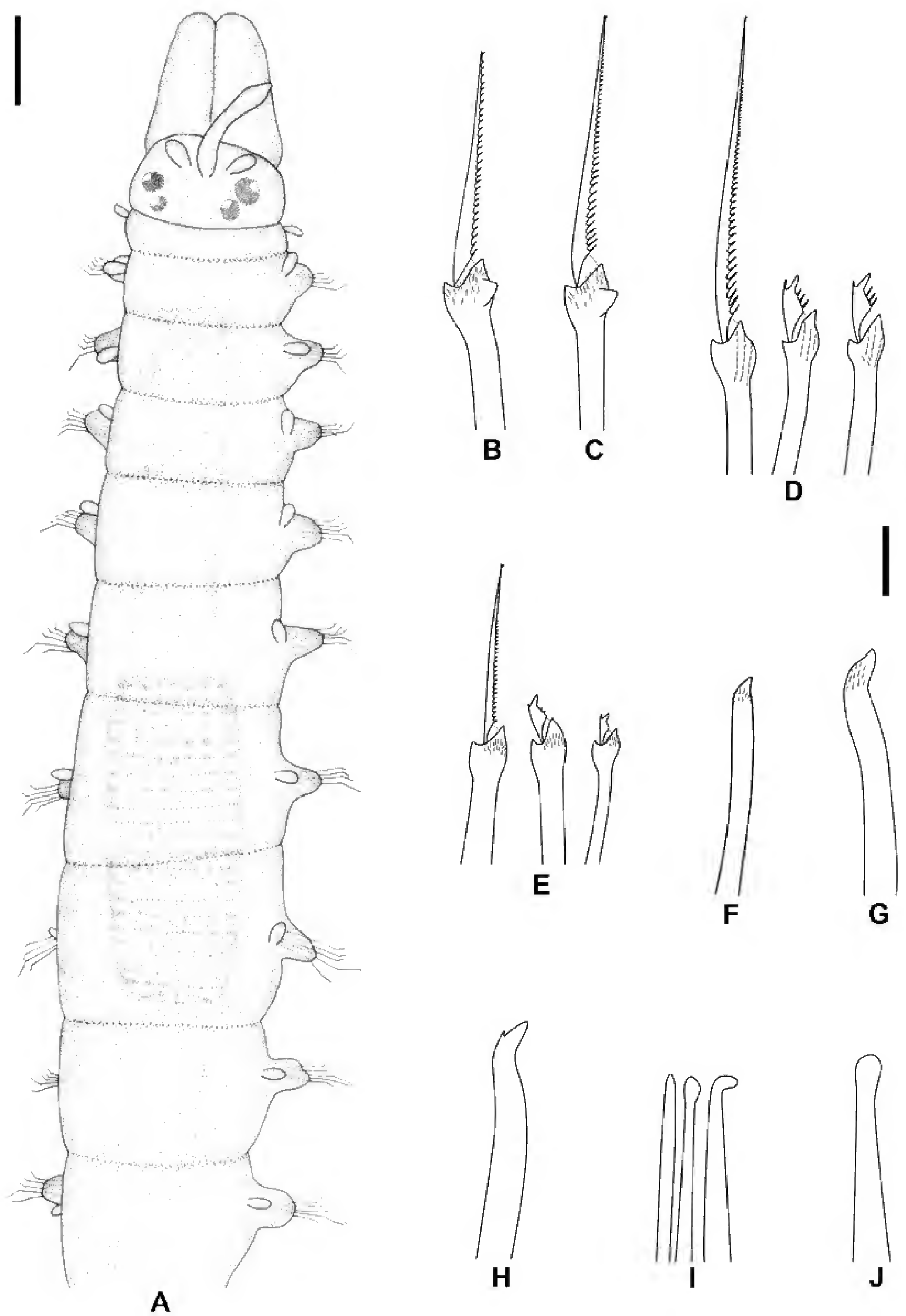

Figure 1. *Exogone cebimar* sp. nov.: A, anterior body, dorsal view; B, spiniger-like chaeta, chaetiger 1; C, spiniger-like chaeta, chaetiger 2; D, compound chaetae, anterior and midbody; E, compound chaetae, posterior body; F, dorsal simple chaeta, anterior body; G, dorsal simple chaeta, posterior body; H, ventral simple chaeta; I, aciculae, anterior body; J, acicula, posterior body. Scale bars: A = 100 μm, B–H = 10 μm.

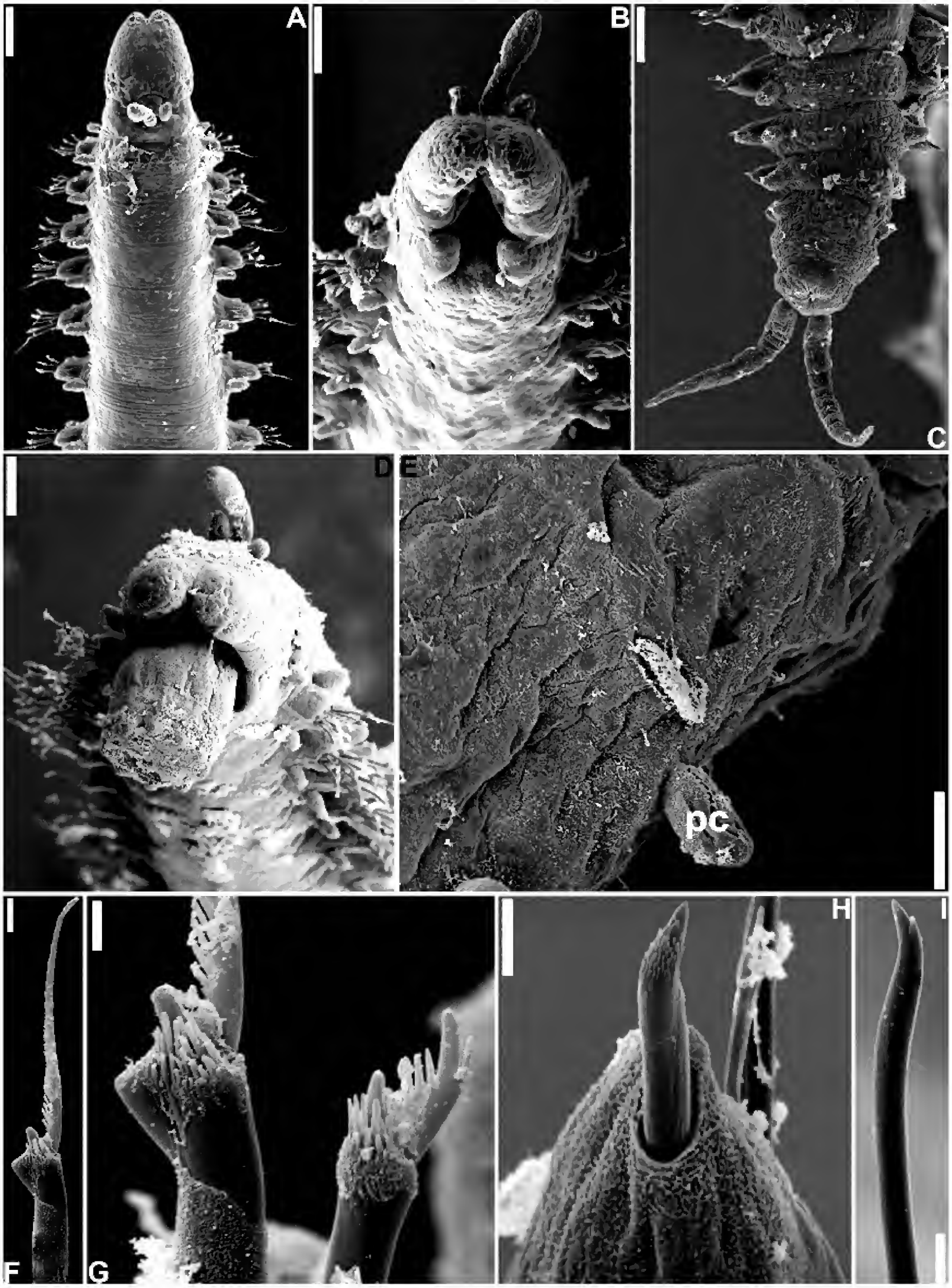

Figure 2. *Exogone cebimar* sp. nov., SEM: A, anterior body, dorsal view; B, anterior body, ventral view; C, posterior body and pygidium, dorsal view; D, anterior body, frontoventral view; E, peristomium, right-hand side dorsolateral view (arrow pointing to nuchal organ; 'pc', peristomial cirrus); F, spiniger-like chaeta, chaetiger 2; G, detail of shafts, spiniger-like chaeta and falciger, chaetiger 2; H, dorsal simple chaeta, posterior body; I, ventral simple chaeta. Scale bars: A = 70 μm, B = 48 μm, C = 42 μm, D = 50 μm, E = 20 μm, F = 3.6 μm, G = 1 μm, H = 5 μm, I = 4 μm.

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A review of the occurrence and ecology of dense populations of *Ditrupa arietina* (Polychaeta: Serpulidae)

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Abstract

Hartley, J.P. 2014. A review of the occurrence and ecology of dense populations of *Ditrupa arietina* (Polychaeta: Serpulidae). *Memoirs of Museum Victoria* 71: 85–95.

Dense populations of the free-living serpulid *Ditrupa arietina* were first recorded to the west and north of the Shetland Isles in the 1920s and have since been reported from the Celtic and North Seas, the Armorican shelf, the Mediterranean and the Azores. These dense populations (of many thousands per square metre) numerically dominate the benthic fauna, and the tubes provide sites of attachment for a range of other species. Vacated tubes are also occupied by other animals, and tube fragments can contribute significantly to biogenic carbonate sediments, both Recent and fossil. Dense *Ditrupa* populations have been the subject of detailed autecological research over the last 15 years, but in spite of the apparent ecological importance of the species, it is not reflected in the European Nature Information System (EUNIS) or other North-east (NE) Atlantic habitat classifications. This paper provides a synthesis of the environmental conditions where high densities of *Ditrupa* have been found, with new data from seabed samples and photos. *Ditrupa* appears to occupy different habitats in the NE Atlantic and the Mediterranean, and studies of its morphology and genetics are needed to determine if there is a taxonomic basis to this ecological separation. Although the evidence is sparse, it is concluded that, in the NE Atlantic, dense populations of *Ditrupa* are found in areas where the seabed is periodically disturbed by internal wave action. European and other habitat classification schemes require revision to reflect the areas of occurrence and benthic effects of internal waves.

Keywords polychaete, North-east Atlantic, Mediterranean, faunal assemblage, habitat classification, EUNIS, disturbance, internal waves

Introduction

The free-living serpulid polychaete *Ditrupa arietina* (O.F. Müller, 1776), was described from material probably from Norway or Denmark (ten Hove and Smith, 1990). Until 1990, it was generally considered to have a cosmopolitan distribution; ten Hove and Smith (1990) clarified the taxonomy of the genus and concluded that the distribution of *D. arietina* was boreal to subtropical East Atlantic. The genus is of geological importance, with extensive fossil deposits (see, for example, Dominici, 2001 and Martinell et al., 2012) and makes significant contributions to some Recent biogenic carbonate sands and gravels (see Wilson 1979, 1982). Ecologically, the tubes provide sites of attachment for other taxa, including solitary corals, other serpulids, foraminiferans and bryozoans (see McIntosh, 1923; Wilson, 1976), while vacated tubes are occupied by a range of animals (see Myers and McGrath, 1979; Wilson, 1982). Predators of the species are poorly known, with the exception of flatfish (Rae, 1956) and naticid gastropods, which leave characteristic drill holes (Grey et al., 2005). The species can achieve very high densities (~11,000/m^2) (Grémare et al., 1998) and has been listed as characteristic of some benthic assemblages (e.g. Stephen, 1923; Glémarec, 1969; Labrune et al., 2007a) although it is not listed in any assemblage in EUNIS (European Nature Information System), a pan-European classification of marine, freshwater and terrestrial habitats (http://eunis.eea.europa.eu/about.jsp). This review of the occurrence of dense populations of *D. arietina* and whether it is a characterising species of particular habitat types was prompted by finding the species in great abundance during a regional survey of the northern North Sea, within a faunal assemblage that closely matched that described by Stephen (1923).

Benthic assemblages characterised by high densities of *Ditrupa arietina*

Fig. 1 shows the distribution of records of high densities of *Ditrupa* from the North-east (NE) Atlantic and Mediterranean (where positions or maps were given), and for convenience the records are summarised below by geographic area. Greater emphasis is placed on the NE Atlantic records (including previously unpublished data) as a series of recent papers describe and discuss the occurrence of high densities of *Ditrupa* in the Mediterranean.

NE Atlantic records of high densities of *Ditrupa arietina*

The first quantitative benthic study to report high densities of *Ditrupa* was by Stephen (1923, as *Ditrupa subulata* = *arietina*). He sampled extensively across the central and northern North Sea and to the west of Scotland using a Petersen grab and an ~1.5mm sieve. Stephen (1923) distinguished a series of faunal community types, including a *Ditrupa* community to the north and west of Shetland with two variations: pure *Ditrupa* (at up to 720/m^2 cited in the text and 360/m^2 listed in Table VI) described as "very barren with few other forms being found where it occurs", and *Ditrupa* with *Ophiura affinis*, described as "a mingling of *Ditrupa subulata* with the *Ophiura affinis* community". McIntosh (1869) had earlier noted *Ditrupa* (as *Ditrypa*) to be "abundant" off Shetland, from dredgings made around the islands in 1867 and 1868, but without quantification. Since he lists some other species as "very abundant" it is here considered that these densities of *Ditrupa* were not exceptional. McIntosh (1923) also recorded the species as abundant, but as he cited Crawshay (1912), who reported a single specimen from the western English Channel, again it is concluded that the densities were not particularly high.

Le Danois (1948) described a "facies à Dentales" from around the shelf edge of the Celtic Sea and off North Gascony with the scaphopod *Dentalium* and solitary coral *Caryophyllia* listed as characteristic taxa. In his list of the fauna of the shelf edge facies, *Ditrupa* was included (along with several other serpulid taxa) under 'epifauna'; *Ditrupa* was also included in lists of the principal fauna of the muddy facies of the Atlantic slope and of the semi-abyssal zone. In Supplement 1 to Le Danois's book, *Ditrupa* was not indicated to be either a facies characterising or a most important species, suggesting that it had not been found in great abundance.

Glémarec (1969) summarised the benthic faunal communities present off the North Gascony coast and mapped (his Fig. 1) a broad area of the outer Armorican shelf as comprising *Ditrupa* sands ("sables à alènes"). These were described as "sables roux à pointes d'alènes" with a median diameter of 270–400 μm and a zoogenic calcium carbonate content of >50%. Glémarec (1969) included (his Fig. 2) a seabed photo showing numerous *Ditrupa* tubes but did not give densities. The community was considered equivalent to the facies "à Dentales de la bordure continentale" distinguished by Le Danois (1948). This could be a suggestion that Le Danois's "Dentales" also included *Ditrupa*, although the difference could also reflect a major increase in *Ditrupa* densities in the decades between the surveys. Glémarec (1973), in his consideration of the European North Atlantic shelf benthic communities, included similar information on the *Ditrupa arietina*/*Dentalium entalis* community (of open sea étage fine sands) to that in Glémarec (1969) and Le Danois (1948) but again without densities; surprisingly, Stephen's (1923) report of a *Ditrupa* community widespread off Shetland was not cited.

A survey of the benthic fauna of the Celtic Sea was undertaken in 1974 and 1975 with the results given in a limited circulation report (Hartley and Dicks, 1977) and mollusc records published by Hartley (1979). *Ditrupa* was present at 20 of 86 stations sampled, with very high densities found at three sites off south-west (SW) Ireland sampled in May/June 1975 from the *RV Challenger*, summarised in table 1. The trawl at station C27 recovered some 14,800 live *Ditrupa*, and previously unpublished quantitative data from two 0.1m^2 grab samples from station C26 are given in table 2, where *Ditrupa* comprised 84% of the fauna retained on a 1mm mesh.

The fauna from the trawl at station C31 suggests some temporal persistence of the population, with a mix of living *Ditrupa* and vacated tubes occupied by other species or living on them. Of the 624 *Ditrupa* tubes retained by the trawl, 173 (28%) contained live *D. arietina*, 214 (34%) contained the amphipod *Siphonoecetes striatus* and 22 (4%) contained the sipunculan *Phascolion strombus*. In total, over 50% of empty tubes were occupied by other taxa, and the tubes frequently had the coral *Caryophyllia smithii* (35, 6%) or the serpulid *Hydroides norvegica* (18, 3%) attached. These observations and others (e.g. Gambi and Jerace, 1997; Morton and Salvador, 2009 ; Ferrero-Vicente et al., 2014) emphasise the importance of living and empty *Ditrupa* tubes as a habitat. The high densities of *Ditrupa* at some Celtic Sea stations were also noted by Dicks and Hartley (1982); they commented that the reasons for the establishment of such high-density, low-diversity communities in shelf depths were unclear.

Wilson (1982) suggested that *Ditrupa arietina* was the most important indicator species characteristic of the rippled sands of the ocean-facing outer continental shelf in the weak current areas of the western Celtic Sea and to the west of Brittany and Scotland. He noted that data on the density and distribution of *Ditrupa* were sparse, although to the west of Scotland the species occurred in discrete patches with densities of up to 1600/m^2. Wilson et al. (1983) obtained "several thousand live *Ditrupa*" from an anchor box dredge sample taken in September 1979 from a sand patch ~59 km west of the Hebrides. Dyer et al. (1982) noted and illustrated with a seabed photo taken in 1978, high densities of *Ditrupa* to the west of Shetland and indicated that *Ditrupa* and the echinoid *Cidaris* were characteristic of the area. Cranmer et al. (1984) also reported that *Ditrupa* was common and locally abundant in the area.

A stratified random regional survey of the East Shetland Basin of the North Sea was undertaken in July 2007 with samples obtained by 0.1m^2 Day grab from 86 stations (Hartley Anderson Ltd, 2008). *Ditrupa* was present at 24 stations, typically at low densities, but at stations 26, 28 and 40 it numerically dominated the fauna retained on a 1mm mesh (see tables 3, 4, 5 and 6 and fig. 2).

The two stations (26 and 28) with the highest densities of *Ditrupa* are species-rich and have an abundant fauna, with above survey average S (110 taxa) and N (909 individuals/0.1 m^2, n = 69). Thus the fauna is not high density, low diversity, as found in the NW Mediterranean (H' of ~2.5, Labrune et al., 2007a) and in the Celtic Sea (table 2). This suggests that in the northern North Sea at least, dense populations of *Ditrupa* can establish (by larval settlement and/or post larval redistribution) in the presence of an existing and diverse fauna, and in the presumed absence of significant physical disturbance. In addition, the station 26 and 28 results indicate that the presence and feeding activities of *Ditrupa* do not lead to a significant loss of diversity in the other fauna. The numerically important taxa listed in tables 5 and 6 show a good degree of commonality

Table 1. Details of high *Ditrupa* density stations in the Celtic Sea (Hartley and Dicks, 1977).

Station number	Sampling gear	Location	Depth (m)	Sediment type (visual observation)
C26	Agassiz trawl 0.1 m^2 Day grab (x2)	50°51'48"N 08°29'18"W	113	Muddy sand
C27	Agassiz trawl	50°57'54"N 08°42'0"W	110	Mud, sand, gravel, shells
C31	Agassiz trawl 0.1 m^2 Day grab	50°42'12"N 09°17'48"W	126	Sand, shells

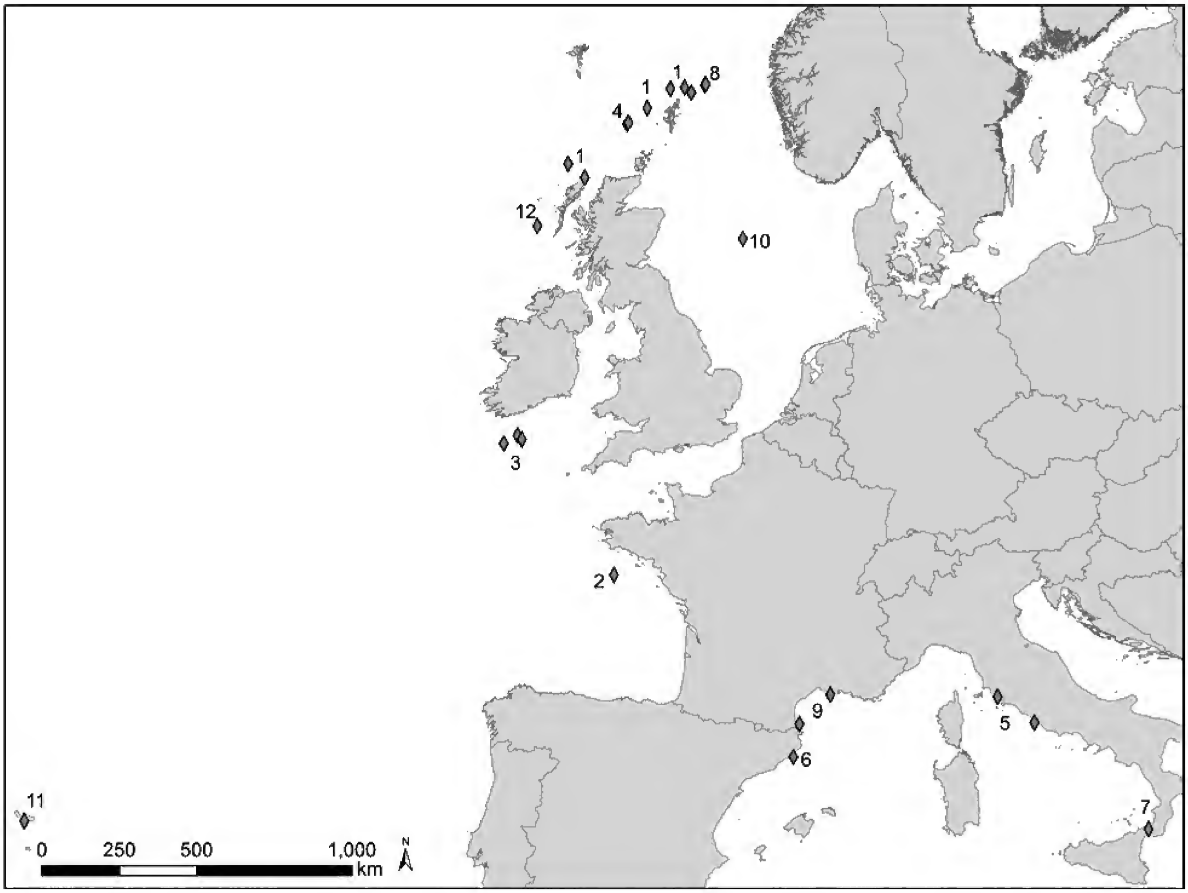

Figure 1. Records of high densities of *Ditrupa* from the North-east Atlantic and Mediterranean Sea. 1, Stephen (1923). 2, Glémarec (1969). 3, Hartley and Dicks (1977). 4, Dyer et al. (1982). 5, Gambi and Giangrande (1986). 6, Grémare et al. (1998). 7, Cosentino and Giacobbe (2006). 8, Hartley Anderson Ltd (2008). 9, Labrune et al. (2007a). 10, Gardline (2009). 11, Morton and Salvador (2009). 12, Wilson et al. (1983).

between stations (particularly stations 26 and 28, which clustered at >70% similarity in classification analysis) (Hartley Anderson Ltd, 2008). The use of 0.5mm and 1mm sieves results in some differences in the lists of numerically important taxa, but regardless of mesh size and the presence of juveniles, especially in the 0.5mm sieve data, the faunal dominance of *Ditrupa* is evident at stations 26, 28 and 40. The occurrence in abundance of the solitary coral *Caryophyllia smithii* where numerous *Ditrupa* tubes are present agrees with the findings of Wilson (1976).

Table 2. Total fauna from two $0.1m^2$ grab samples from Celtic Sea survey station C26 (see Hartley and Dicks, 1977; nomenclature has been updated).

Station C26	Grab 1	Grab 2
Ditrupa arietina	377	741
Myriochele spp. agg.	41	52
Echinoidea juv.	2	19
Anthozoa juv.	3	10
Echinocyamus pusillus	2	9
Siphonoecetes striatus	1	9
Ophiura affinis	–	9
Phaxas pellucidus	2	7
Ophiuridae juv.	–	8
Aspidosiphon muelleri	2	5
Abra nitida	2	5
Hilbigneris gracilis	4	1
Amphictene auricoma	1	2
Owenia fusiformis	2	1
Amphipoda indet.	1	2
Ampelisca indet.	–	3
Ampharetidae juv.	–	3
Magelona sp.	2	–
Paranymphon spinosum	–	1
Atelecyclus rotundatus	1	–
Corbula gibba	–	1
$\Sigma/0.1\ m^2$	446	885

Seabed photos taken at a site at 75 m depth in the central North Sea showed numerous *Ditrupa* tubes lying on the sediment surface (Gardline 2009, site JRP). Grab samples at the site indicated a *Ditrupa* density of $390/m^2$ in moderately sorted fine sand with a mean grain size of 157 μm and a silt/clay content of 6.7% (ERT, 2009). This is considered to be a small patch of *Ditrupa*, as photos and samples from 16 other stations within 8 km showed the species to be absent or rare, which is consistent with the results of numerous macrofaunal surveys in the region (Rees et al., 2007, UK Benthos database and unpublished data).

Morton and Salvador (2009) reported *Ditrupa* at ~100–250 m depth off the Azores, and as a significant component of the fauna at depths of ~200 m; the samples were taken by dredge and quantitative density information was not included. They highlighted the different depth zones of *Ditrupa* occurrence between the Mediterranean and the Azores and suggested this may be related to differences in light penetration or food availability, or other factors such as sediment type or disturbance. Morton and Salvador (2009) also illustrate (their Fig. 4A[1]) and describe the live position of the worm (with the majority of the tube buried in the sediment) and indicate that, when placed on sediment, worms attempt to re-burrow. This contrasts with the range of published seabed photographs showing tubes lying at the sediment surface and would call into question the findings of Guizien et al. (2010) of *Ditrupa* spatial redistribution caused by swell-induced bed load transport.

Ellis et al. (2002, 2013) in a regional beam trawl study of the epifauna of the Celtic Sea noted that *Ditrupa* was very abundant at a number of sites and that it "was abundant off south-western Ireland at depths of 102–305 m". Ellis et al. (2002) listed *Ditrupa* in the dominant fauna associated with a *Pagurus prideaux–Porania pulvillus* assemblage of the southern Celtic Sea; a similar assemblage and its occurrence was described by Ellis et al. (2013), although *Ditrupa* was not listed in the dominant fauna.

Selected UK and North Sea areas where *Ditrupa arietina* is absent

Based on detailed regional surveys, it is apparent that *Ditrupa* is absent from some areas, such as the southern North Sea (Degraer et al., 2006; Daan and Mulder, 2006; Diesing et al., 2009; Tappin et al., 2011) and the Irish Sea (Bruce et al., 1963; Mackie et al., 1995; Robinson et al., 2009; Hartley Anderson Ltd, 2009). These areas coincide with non-stratified waters or areas of shallow stratification and suggest that in the North Atlantic *Ditrupa* is restricted to Glémarec's (1973) open sea étage, where annual thermal variations are small; this is in apparent contrast to the situation in the NW Mediterranean, where dense populations of *Ditrupa* are found in shallow 10–30 m coastal waters where bottom water temperatures have an ~10°C annual variation (Charles et al., 2003, their Fig. 9). Regional epifaunal surveys of the North Sea undertaken using beam trawls (Jennings et al., 1999; Callaway et al., 2002) did not report *Ditrupa*, even in areas of known occurrence; this is believed to reflect the sampling method.

Mediterranean records of high densities of *Ditrupa arietina*

Ditrupa was historically considered to be an uncommon species in the Mediterranean, although a dramatic increase in abundance along the NW Mediterranean coast occurred around 30 years ago. Grémare et al. (1998) reported high densities ($>1000/m^2$) of *Ditrupa arietina* at all sites sampled in surveys off the Catalan coast carried out in the 1990s, with maximum densities of $11{,}086/m^2$, accounting for as much as 79% of total macrofaunal abundance and biomass. *Ditrupa* was predominantly found in depths of between 20 and 30 m in well-sorted fine sands and muddy sands. Grémare et al. (1998) concluded that *Ditrupa* abundance had recently increased all along the Catalan coast (as there were few reports of the species in the area before 1970) and that the increase was not due to sediment instability but rather to a reduction in silt/clay in the sediment due to increased frequency of easterly storms. Sardá et al. (2000) also reported a significant increase in *Ditrupa* numbers in a shallow water area off the mouth of the Tordera River (Catalan coast) following the removal of sand by suction dredging for beach replenishment. The dredging defaunated the sediments and changed their grain size composition; fine sands redistributed over the winter after cessation of dredging and in spring were densely colonised by a range of species, including *Ditrupa*, which attained densities of $\sim 2800/m^2$.

Table 3. Details of the highest *Ditrupa* density stations in the northern North Sea (Hartley Anderson Ltd, 2008).

Station number	**Location**	**Depth (m)**	**Sediment type**	**S[a]**	**N[a]**	**H'(log_2)**	***Ditrupa* % of total fauna**
26	61.216492° N 0.795038° E	166	Very poorly sorted fine sand	119	1093	4.9	43 (1 mm) 22 (0.5 + 1 mm)
28	61.236048° N 0.855043° E	167	Very poorly sorted fine sand	128	1222	4.8	49 (1 mm) 26 (0.5 + 1 mm)
40	61.024875° N 0.750020° E	156	No data	85	472	5.1	23 (1 mm) 12 (0.5 + 1 mm)
42	60.999035° N 0.618103° E	151	Poorly sorted fine sand	90	615	5.2	2 (1 mm) 3 (0.5 + 1 mm)

[a]Numbers from 0.1 m^2 sieved on 0.5mm mesh

Table 4. Sediment characteristics for highest *Ditrupa* density stations in the northern North Sea (Hartley Anderson Ltd, 2008)

Station	**Carbonate %**	**Organic %**	**Mean diameter (μm)**	**Coarse % (>2 mm)**	**Fine % (<63 μm)**	**Silt %**	**Clay %**
26	30.99	0.94	141	2.19	15.86	11.79	4.07
28	30.09	0.73	159	1.64	14.76	11.04	3.72
42	30.43	0.56	240	0.21	6.98	4.67	2.31

Figure 2. Photo of East Shetland Basin survey station 28 sample with numerous *Ditrupa* tubes; also visible are the solitary coral *Caryophyllia smithii* (red arrow) and the foraminiferan *Astrorhiza arenaria* (yellow arrow).

Table 5. The ten most abundant taxa (by rank) in metazoan fauna >0.5 mm at the highest *Ditrupa* density stations in the northern North Sea. Densities are numbers per 0.1 m² (Hartley Anderson Ltd, 2008).

Station 26 ∑1093/0.1 m²		**Station 40 ∑472/0.1 m²**	
Ditrupa arietina	243	*Ditrupa arietina*	56
Minuspio cirrifera	146	*Aricidea wassi*	49
Ophiura affinis	100	*Minuspio cirrifera*	47
Spiophanes kroyeri	45	*Spiophanes kroyeri*	41
Euchone sp. 1	44	*Myriochele* spp. agg.	26
Echinocardium juv.	44	*Owenia fusiformis*	17
Aricidea wassi	28	*Spiophanes bombyx*	14
Eclysippe cf. *vanelli*	28	*Echinocyamus pusillus*	14
Axinulus croulinensis	27	*Poecilochaetus serpens*	11
Glycera lapidum	21	*Glycinde nordmanni*	10
		Aonides paucibranchiata	10
		Ampharetidae juv.	10
		Echinocardium juv.	10
Station 28 ∑1222/0.1 m²		**Station 42 ∑615/0.1 m²**	
Ditrupa arietina	322	*Minuspio cirrifera*	64
Minuspio cirrifera	174	*Myriochele* spp. agg.	61
Ophiura affinis	94	*Aricidea wassi*	44
Echinocardium juv.	42	*Ophiura affinis*	44
Spiophanes kroyeri	35	*Echinocardium* juv.	35
Eclysippe cf. *vanelli*	31	*Spiophanes kroyeri*	33
Ampharetidae juv.	28	*Owenia fusiformis*	33
Axinulus croulinensis	25	*Euchone* sp. 1	19
Mugga wahrbergi	24	*Ditrupa arietina*	18
Polynoidae juv.	17	*Echinocyamus pusillus*	18
Myriochele spp. agg.	16		
Paraonides sp. 1	16		
Euchone sp. 1	16		

Table 6. The ten most abundant taxa (by rank) in metazoan fauna >1.0 mm at the highest *Ditrupa* density stations in the northern North Sea. Densities are numbers per 0.1 m² (Hartley Anderson Ltd, 2008).

Station 26 ∑562/0.1 m²		**Station 40 ∑242/0.1 m²**	
Ditrupa arietina	243	*Ditrupa arietina*	56
Ophiura affinis	47	*Spiophanes kroyeri*	27
Echinocardium juv.	42	*Owenia fusiformis*	15
Spiophanes kroyeri	31	*Spiophanes bombyx*	13
Minuspio cirrifera	20	*Echinocardium* juv.	10
Euchone sp. 1	17	*Echinocyamus pusillus*	9
Eclysippe cf. *vanelli*	14	*Minuspio cirrifera*	8
Caryophyllia smithii	12	Ampharetidae juv.	7
Glycera lapidum	6	*Euchone* sp. 1	6
Praxillella affinis	5	*Polydora* sp.	5
Polycirrus arcticus	5	*Urothoe elegans*	5
Cirolana borealis	5	*Yoldiella philippiana*	5
Eudorella truncatula	5		
Yoldiella philippiana	5		
Station 28 ∑659/0.1 m²		**Station 42 ∑257/0.1 m²**	
Ditrupa arietina	322	*Echinocardium* juv.	30
Ophiura affinis	55	*Owenia fusiformis*	27
Echinocardium juv.	42	*Ophiura affinis*	27
Spiophanes kroyeri	24	*Myriochele* spp. agg.	16
Caryophyllia smithii	16	*Spiophanes kroyeri*	15
Minuspio cirrifera	15	*Euchone* sp. 1	12
Eclysippe cf. *vanelli*	13	*Echinocyamus pusillus*	12
Polycirrus arcticus	9	*Minuspio cirrifera*	8
Yoldiella philippiana	9	*Chone longocirrata*	8
Ampharetidae juv.	7	*Spiophanes bombyx*	7
Euchone sp. 1	7	*Pseudopolydora paucibranchiata*	7
		Urothoe elegans	7

Medernach et al. (2000) investigated the ecology of these dense populations of *Ditrupa* and reported the species has a 2-year life span, starts breeding in its first year, has two spawning periods in a year, a planktonic larval stage lasting ~6 weeks, with high larval mortality on initial benthic settlement. Charles et al. (2003) extended these studies and found that settling larvae do not show sediment grain size selectivity, and concluded that the observed spatial heterogeneity in the density and structure of adult populations was mainly due to post-settlement processes. Charles et al. (2003) indicate a planktonic larval stage of 3 weeks (abstract) and ~4–5 weeks (text).

Labrune et al. (2007a), in a regional scale survey of the Gulf of Lions (NW Mediterranean), found from cluster analysis that *Ditrupa* was the numerically dominant polychaete in cluster I, comprising sands (fine to very fine sands with ~10% silt/clay from their Fig. 4) in 10–20m depth (average density of 616/m²) and one of the dominants (average density of 100/m²) in cluster IIa (stations in depths of 30 m in the west of the survey area, fine sands with ~20% silt/clay). Labrune et

al. (2007b) revisited the conclusions of Grémare et al. (1998) on the causes of increased *Ditrupa* abundance and proposed that they were in fact due to greater sediment stability linked to a reduction in the frequency of storms. Guizien et al. (2010) expanded the studies of these *Ditrupa* populations with field and lab flume experiments to investigate the hydrodynamic mobility of the animals in swell-induced currents. They found that normal tidal currents were not sufficient to transport animals with tubes >6 mm, but that moderate swell-induced currents could result in bed load transport of tubes of up to 25mm length. Field sampling before and after a swell event indicated hydrodynamic redistribution of animals, with significant losses or gains in densities of different size classes at some stations sampled; these changes were not linked to larval recruitment but to the translocation of adults. Guizien et al. (2010) considered that *Ditrupa* was epifaunal, without organs to allow burrowing or surface movement, was tolerant of sediment disturbance, and in shallow waters may not strictly be a sedentary species.

Dense populations of *Ditrupa* were reported from the Tyrrhenian Sea by Gambi and Giangrande (1986) and from its southeastern boundary (the Strait of Messina) by Cosentino and Giacobbe (2006). Gambi and Giangrande (1986) sampled around the mouths of the Rivers Tiber and Ombrone and listed *Ditrupa* as a characterising species in two station clusters: cluster C, comprising mixed sediments in water depths of 15–30 m off the Tiber, and cluster A, including fine and very fine sands in 5–10 m off the Ombrone. The samples were taken with a Charcot dredge, and their data are therefore considered semi-quantitative; the abundance data in their Table 1 are without area units. Cosentino and Giacobbe (2006) report high densities of *Ditrupa* (>500/0.25 m^2) in muddy sands (~20% mud) in depths of 35–45 m, where it made up nearly 80% of the polychaete and mollusc fauna. They variously describe the species as being eurytopic (an indicator of high sedimentation rates) and mud-tolerant. Cosentino and Giacobbe (2006) suggest several possible causes for the high *Ditrupa* density, including episodic high sediment load inputs as a result of terrestrial floods, and sediment disturbance/induced instability from pipeline installation. They note the transitory nature of the dense *Ditrupa* population found in their 1992 survey, with declines in abundance in the 1993 and 1995 surveys and an absence of living *Ditrupa* or dead tubes in 1999; this suggests that significant sediment movement(s) had occurred in the area, since complete empty tubes would be expected to endure for several years before fragmenting.

Discussion

The enigmatic discrepancies in the patterns of distribution and abundance of *Ditrupa* between the NE Atlantic and the Mediterranean raise a number of questions. In the NE Atlantic, the species has a wide distribution in waters that strongly stratify thermally (and is apparently absent from waters that do not and in more enclosed basins), with high densities typically found on ocean-facing outer continental shelves and upper slopes. In the Mediterranean, *Ditrupa* was considered uncommon until about 30 years ago but since then it has been widely recorded as a (or the) faunal dominant in shallow waters (typically ~10–30 m depth) of the NW Mediterranean and Tyrrhenian Sea.

This apparent ecological difference may point to the presence of two or more cryptic species, or the presence of an unrecognised introduced species in the Mediterranean. *Ditrupa* tube shape and free-living habit are distinctive and in routine surveys tend to be used for identification without examining the morphology of the worm inside. The Mediterranean examples examined by ten Hove and Smith (1990, from 40–50 m depth in the Baie de Cavalaire) consisted of empty tubes. Ten Hove and Kupriyanova (2009) note that the colour of the animals may be useful for serpulid species discrimination in the field but caution that there is inter- and intraspecific variability and that colour is rapidly lost in fixed material. Tantalisingly, there appears to be a difference in living *Ditrupa* branchial crown colouration from off Madeira (pallid in Fig. 1A in ten Hove and Kupriyanova, 2009) and those from the NW Mediterranean illustrated by Guizien et al. (2010, with red spots in their Fig. 1a). Investigations of the comparative morphology and genetics of specimens from the shallow Mediterranean and deeper NE Atlantic are now needed to resolve this enigma. The *Ditrupa* sequences currently in GenBank are all derived from Mediterranean material (from Banyuls, Kupriyanova et al., 2006; Kupriyanova and Rouse, 2008; and from Collioure, Lehrke et al., 2007).

An alternative explanation for the apparent ecological difference and absence from northern non-stratified waters is the thermal tolerance of the species, possibly in relation to winter minimum temperature, which in the southern North Sea can be ~4°C. Annual temperature variation seems an unlikely candidate, since Charles et al. (2003) illustrated an ~10°C range for the NW Mediterranean, which is similar to that recorded for the Celtic Sea.

There is a paucity of published detail on the sediment types occupied by dense *Ditrupa* populations, with a reported range from medium through very fine sands with a variable proportion of mud (<63 μm), to muds. However, sediment type may not be a key determining factor, based on the findings of Charles et al. (2003) that settling larvae do not show sediment grain size selectivity, and the density and structure of adult populations was mainly due to post-settlement processes.

Areas where dense populations of *Ditrupa* have been reported are subject to periodic sediment disturbance; in the shallow waters of the Mediterranean such disturbance has been attributed to storms (Grémare et al., 1998; Guizien et al., 2010), other physical processes, including strong tides, floodwaters and seismic activity (Cosentino and Giacobbe, 2006), or human activities such as sand extraction (Sardá et al., 2000) and pipeline installation (Cosentino and Giacobbe, 2006). In contrast, the areas of the NE Atlantic where abundant *Ditrupa* have been found are in water depths where storm-wave-induced oscillatory currents would not result in sediment disturbance (Draper, 1967) or be affected by strong tides, flood waters or seismic activity. Trawling is an additional source of sediment disturbance that could facilitate the establishment of high population densities of *Ditrupa* by disrupting elements of the existing benthic community.

However, in view of the extensive and long-term trawling that has occurred in the North Sea and the general absence of records of abundant *Ditrupa* in numerous benthic surveys, this does not appear to a major factor.

A source of sediment disturbance that does not seem to have been considered in previous discussions of dense *Ditrupa* populations is internal waves. These energetic phenomena are of global occurrence in waters with strong density gradients, and Jackson (2004) includes examples from all the areas considered earlier in this paper except the Tyrrhenian Sea. However, Nash and Moum (2005) identified river plumes as a source of internal waves, which indicates they could affect the areas studied by Gambi and Giangrande (1986). Pomar et al. (2012, see also Pomar et al., 2013) reviewed the major effects of internal waves on sediment mobilisation, structures and the sedimentary record. In the Celtic Sea (and Armorican shelf), the areas of high *Ditrupa* abundance appear to correspond with those influenced by internal waves, which occur during the summer months (July to September), when a well-developed thermocline is present (Pingree and Mardell, 1981; Jackson, 2004). Internal waves near the Celtic Sea shelf break have been observed to amplify spring barotropic tidal currents to in excess of 100 cm s^{-1} and may be important in modifying sediment transport rates (Heathershaw, 1985). As the Celtic Sea internal waves are seasonal, the sediment disturbance they cause may be an important factor in the establishment of dense populations of *Ditrupa*. Sediment disturbance may promote high *Ditrupa* densities in two ways: disruption of the established benthic fauna, allowing successful recruitment of large numbers of *Ditrupa* larvae; and through post-settlement redistribution and concentration in areas of deposition. The variability of internal wave occurrence, intensity and depth of impingement on the seabed (reflecting the variability of the seasonal and permanent pycnoclines) can be conjectured to explain why dense populations of *Ditrupa* near the shelf break appear to occur in patches rather than a continuous band. However, the Celtic Sea areas where *Ditrupa* occurs in abundance appear to be consistent with the near shelf break, where mixing by internal tide breaking results in a phytoplankton community dominated by picoeukaryotes and other larger phytoplankton, and which is distinct from that of adjacent oceanic and shelf areas (Green et al., 2008; Sharples et al., 2009). Sharples et al. (2009) indicate that the internal tide occurs regularly throughout the stratified season (April to September) and propose links to it, and its effects, on the timing of fish spawning, larval feeding, and the export of particulate organic matter out of the photic zone. Therefore, a possible alternative explanation for *Ditrupa* abundance in parts of the Celtic Sea is the seasonally enhanced food supply (plankton-derived particulate organic matter), which the life history of the species allows it to exploit effectively through post-larval redistribution into areas of particulate organic matter settlement. Charles et al. (2003) indicate that in the NW Mediterranean, the great bulk of *Ditrupa* larval settlement occurs in April to May (the spawning period in the NE Atlantic), and the environmental cues triggering it are not known, but, based on water temperatures, later spawning in the Celtic Sea can be conjectured.

Other NE Atlantic shelf edge areas and islands where dense populations of *Ditrupa* have been reported also develop seasonal thermoclines, have permanent thermoclines or have pycnoclines at the interface between different water masses. Therefore, sediment disturbance through internal wave breaking is proposed as a likely major contributory factor to the occurrence of such populations, potentially also linked to seasonal enhancement of plankton-derived food supply. The maps illustrating shelf edge surface chlorophyll peaks around the British Isles in Pingree and Mardell (1981, their plates 1 and 2) and Sharples et al. (2009, their Fig. 1) indicate areas of search for dense *Ditrupa* populations in future sampling exercises.

There do not appear to be any published time-series of benthic surveys of NE Atlantic areas where dense *Ditrupa* populations have been found. As a consequence, much of this discussion has relied on extrapolation from shallow-water Mediterranean evidence, which may not be applicable to deeper waters of the outer shelf and upper continental slope. Repeated sampling is required to better document the ecology of NE Atlantic *Ditrupa* populations and their role in characterising the benthic assemblages of the continental shelf. The different sampling methods used are a confounding variable in perspectives on benthic assemblages with *Ditrupa*. In particular, the sieve mesh used influences perceptions of the species composition and relative diversity of associated fauna (compare Stephen's (1923) results from a ~1.5 mm sieve with those from 1.0mm and 0.5mm meshes in tables 5 and 6 above). Similarly, trawl samplers may not retain *Ditrupa* unless they clog with sediment or have a fine mesh liner; thus it is uncertain if *Ditrupa* was not common in the wide area of the northern North Sea surveyed by Basford et al. (1989) or just not sampled. Eleftheriou and Basford (1989) did not include uncommon taxa in the interpretation of their regional grab survey of the northern North Sea, and since *Ditrupa* was not mentioned, it is assumed that it was not abundant in their samples. Seabed photography is valuable in documenting *Ditrupa* presence and density, ideally augmented with physical sampling to allow distinction between live animals and empty tubes. It is not known whether the difference in relative faunal diversity apparent between samples from the Celtic Sea (table 2) and northern North Sea (table 3) is real or a reflection of the low number of samples and differences in sample processing methods in the field; further sampling is required to enable valid comparisons to elucidate this.

This review aimed to conclude whether benthic assemblages dominated by *Ditrupa* were sufficiently consistent in their occurrence to warrant inclusion in habitat classifications, or alternatively, whether they were ephemeral and thus rightly excluded. For the NE Atlantic, the weight of evidence (albeit limited) suggests that dense populations of *Ditrupa* occur with sufficient regularity in areas with well-bounded depth and hydrodynamic conditions that the assemblage should be included in habitat classifications. Such classification schemes would benefit from revision to take account of internal waves as a significant source of bed shear stress, sediment disturbance and trophic modification in the deep circalittoral and upper slope, areas currently defined as where the seabed is not affected by waves. Considerable effort

has recently been expended to improve European habitat classifications for use in marine spatial planning and identification of marine protected areas (West et al., 2010; McBreen et al., 2011; Cameron and Askew, 2011), but none makes any reference to internal waves. This is remarkable since they have long been documented, together with their links to ecologically important habitats such as cold water corals (Frederiksen et al., 1992) and to sediment disturbance (Heathershaw, 1985; Hosegood and van Haren, 2004).

In addition, the ecology of *Ditrupa* presents a number of interesting questions. Is its free-living habit (in contrast to most other serpulids) an adaptation that allows colonisation of sedimentary areas, particularly those subject to periodic hydrodynamic disturbance, with the robust elephant tusk shaped tube allowing successful post larval and adult animal redistribution via bed-load transport, and facilitating both filter and surface deposit feeding? Is the operculum used by an animal to allow some repositioning or as an anchor in periods of high bed shear stress? Does the resulting spatially and temporally variable patchwork of *Ditrupa* densities hinder the establishment of high densities of predators such as naticid gastropods, or parasites?

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Polychaete diversity in the estuarine habitats of Términos Lagoon, southern Gulf of Mexico

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Abstract

Hernández-Alcántara, P., Cortés-Solano, J.D., Medina-Cantú, N.M., Avilés-Díaz, A.L. and Solís-Weiss, V. 2014. Polychaete diversity in the estuarine habitats of Términos Lagoon, Southern Gulf of Mexico. *Memoirs of Museum Victoria* 71: 97–107.

In order to establish the status of the biodiversity of the polychaete fauna inhabiting the soft bottoms in the largest lagoon-estuarine system from the southern end of the Gulf of Mexico, Términos Lagoon, we collected and identified 3,398 specimens belonging to 119 species and 33 families of polychaetes. The soft bottom fauna was then compared with records of polychaetes collected in other habitats in the lagoon such as seagrass beds and mangroves. In all, 190 species from 34 families of polychaetes previously recorded there were taken into account. The families Nereididae (20 spp.), Spionidae (15 spp.) and Syllidae (14 spp.) were the most diverse. The soft bottom habitat has by far the largest number of species (119) followed by the seagrass beds and mangroves with 75 and 42 species respectively. Large spatial heterogeneity in polychaete composition was observed, as only 5% of the species (*Melinna maculata*, *Capitella* sp., *Mediomastus californiensis*, *Schistomeringos rudolphii*, *Marphysa sanguinea*, *Alitta succinea*, *Diopatra cuprea*, *Scoloplos treadwelli*, *Prionospio heterobranchia* and *Scolelepis squamata*) were widely distributed in the lagoon. The polychaete fauna living in the mangroves is quite similar to that from seagrasses and soft bottoms ($R_{(ANOSIM)}$ = 0.247 and 0.3 respectively), but the polychaetes in the seagrasses and soft bottoms are clearly different from each other ($R_{(ANOSIM)}$ = 0.622). The 119 polychaete species identified in this study represent a significant increase in the records of biodiversity recorded so far in Términos Lagoon, while the total of 190 species recorded for the whole lagoon represents a larger number than any other recorded for an American tropical estuary.

Keywords Polychaeta, soft bottoms, seagrass beds, mangroves, distribution, Mexico

Introduction

The lagoon-estuarine environments are one of the most productive aquatic systems on earth and constitute important refuges, breeding and feeding grounds for marine and freshwater organisms that commonly live there or visit them, either occasionally or seasonally. These environments play a prominent role for man, due to their biological diversity and the fishing activities that are usually associated. However, the continuing increase of the human populations around these grounds has taken its toll and the negative effects are evident on the flora and fauna of the region (Lotze et al., 2006; Orth et al., 2006). Particularly, Términos Lagoon, one of the largest lagoons of Mexico, has been drastically impacted by human pressure during the last decades, mainly due to shrimp fisheries, urbanization of Carmen Island and deforestation of riverine vegetation for intensive agriculture (Villéger et al., 2010).

Coastal lagoons and estuaries are usually sites with low diversity but high faunal abundance due to their special environmental conditions (Constable, 1999). This is associated with the "minimum species" concept expressed by Remane (1934) to explain that the large variations of environmental factors in those brackish waters exclude many species and thus, the transitional marine-freshwater zones are typically

species poor communities. In these water bodies the biota is characterized by a high level of adaptive evolution to stress and to those environmental variations that distinguish those aquatic systems, especially salinity variations. That is why the implementation of studies dedicated to the knowledge of the biodiversity of these lagoon-estuarine systems is fundamental to create monitoring programs that can help mitigate and control the anthropic effects on that biota.

In Mexico, even if coastal lagoons cover approximately 30-35% of its almost 11,000 km of littorals (Contreras, 1985), their study has not been a priority for benthic specialists. In these systems, polychaetes are typically the main component of the macrofaunal communities (Hutchings, 1998), and they frequently represent more than half the number of species and organisms present in any sample (Blake, 1994; Hutchings, 1998; Olsgard et al., 2003). So, it can be assumed that their abundance and diversity patterns are the most important for understanding the functioning of these systems and are crucial to define the structure, production and general dynamics and health of their benthic communities. The biological processes observed in this group alone, could in fact reflect those of the whole benthos in general (Mackie et al., 1997; Glasby and Read, 1998; Olsgard and Somerfield, 2000).

Even if in the southern Gulf of Mexico those systems are widely represented (623,600 ha) and the polychaetes are recognized as one of their most important benthic components (Hernández-Alcántara and Solís-Weiss, 1991, 1995), so far their presence has only been recorded in eleven, including Términos Lagoon, from the 174 lagoon-estuarine systems recorded in that region. That is why the objective of this study is to establish the biodiversity recorded in the polychaetes of the soft bottoms of one of the largest coastal lagoons of the Gulf of Mexico, Términos Lagoon, and to compare it to the variety of species so far recorded there in seagrasses and mangroves, as a departure point for future monitoring programs of the regional benthic fauna.

Methods

Study area

Términos Lagoon is located at the southern end of the Gulf of Mexico (18° 38'N; 91° 34'W); it is about 70 km long and 30 km wide, including two small tributary lagoons and swamp areas (fig. 1). It was declared "Protected Area of Flora and Fauna of Términos Lagoon" (APFFLT for its initials in Spanish) in 1994, and a Ramsar site in 2004. Depth in the Lagoon averages 3.5 m. Two inlets connect it to the Gulf of Mexico (fig. 1), and the winds, coupled with the prevailing currents, force the seawater inflow through the Puerto Real Inlet (to the east), while the lagoon waters outflow through the Carmen Inlet (to the west) (Yáñez-Arancibia and Day, 2005). Soft bottoms devoid of vegetation are the dominant habitat in the lagoon and cover most of the approximately 2500 km^2 of its total surface while mangroves, dominated by *Rhizophora mangle* (Linnaeus, 1753) are present around most of its edges; dense and extensive prairies of seagrass beds dominated by *Thalassia testudinum* Banks ex König, 1805, are also present in the lagoon, although they are restricted to the south and southeast of the Carmen Island and the southeastern end of the lagoon (fig. 1).

Data analysis

The faunal information presented here is primarily based on the specimens collected in soft bottoms in Términos Lagoon as part of the multidisciplinary project "Joint Environmental Study of Términos Lagoon (JEST)", carried out during 2008-2009 by the "Institut de Recherche pour le développement » (IRD) from France, the Universidad Autónoma Metropolitana-Iztapalapa México and the Universidad Nacional Autónoma de México. The objective of the general project was to compare scientific results obtained some 20 years ago, with new data, and thus establish, the present environmental status and biogeochemical functioning of the Lagoon. To this aim, the faunal data in this study were combined with information about the polychaete species previously reported in seagrasses (Ibañez-Aguirre and Solís-Weiss, 1986; Cruz-Ábrego et al., 1994) and mangroves of the Lagoon (Hernández-Alcántara and Solís-Weiss, 1991, 1995).

The biological samples for this study were taken with a Van Veen (0.06 m^2) or Ekman (0.053 m^2) grab at 24 stations distributed evenly over the soft bottoms of the Lagoon. The faunal information for the seagrass and mangrove areas was taken from published sources and made with a quadrat (0.06 m^2) at 22 stations for the seagrasses and five stations with a corer (25 cm inner diameter, 20 cm penetration in the sediment) for the mangroves. All samples collected in each habitat were washed through a 0.5 mm mesh, to separate the macrofauna and fixed in 4% formalin to be later preserved in 70% alcohol.

The comparison of the information resulting from this study with that coming from the literature was complicated by the different sampling procedures used in each case, so that for comparison purposes, the distribution of species in the three studied habitats was analyzed only as presence/absence information. The faunal list presented here is made using the current names of species as well as the names under which they were reported initially (in parentheses), so that they are readily traceable in the original source. Most species names were verified with the World Polychaeta database (Read and Fauchald, 2013), accessed through the World Register of Marine Species (WoRMS, 2013). Before data processing, the original list was filtered to remove all doubtful records, i.e. those attributed to species whose known world distribution does not correspond to the marine region studied here, or species whose identification was incorrect.

The differences among the polychaete species in the three habitats were evaluated with ANOSIM (analysis of similarity). ANOSIM tests the hypothesis that there are no differences between habitats in the composition of species, by calculating the test statistic R which varies from $R = 0$ (groups indistinguishable from one another) to $R = 1$ (no similarity between groups) (Clarke, 1993). By resampling the data, a probability level can be associated with R; in this case $p< 1\%$ was used to distinguish the different habitats. The ANOSIM test was performed using the Plymouth Routines in Multi-Variate Ecological Research (PRIMER v6) software (Clarke and Gorley, 2006).

Table 1. Distribution of the polychaete species collected in the three habitats of Términos Lagoon (SB: soft bottoms; SG: seagrass beds; M: mangroves).

Family - Species	**Habitat**		
	SB	SG	M
Acoetidae			
Polyodontes lupinus (Stimpson, 1856)	+		
Ampharetidae			
Melinnopsis sp. 1	+		
Isolda bipinnata Fauchald, 1977	+	+	
Melinna maculata Webster, 1879	+	+	+
Melinna palmata Grube, 1870		+	
Amphinomidae			
Hipponoe sp.		+	
Linopherus ambigua (Monro, 1933)			+
Arenicolidae			
Arenicola cristata Stimpson, 1856		+	
Capitellidae			
Capitella sp. (as *Capitella capitata* (Fabricius, 1780))	+	+	+
Capitomastus sp.		+	
Mediomastus californiensis Hartman, 1944	+	+	+
Notomastus hemipodus Hartman, 1945 (as *Notomastus luridus* Verrill, 1873		+	
Notomastus sp.		+	
Rasghua sp.	+		
Cirratulidae			
Aphelochaeta sp.	+		
Caulleriella alata (Southern, 1914)			+
Caulleriella bioculata(Keferstein, 1862)		+	
Timarete filigera (Delle-Chiaje, 1828) (as *Cirriformia filigera* (Delle-Chiaje, 1828))		+	
Timarete tentaculata (Montangu, 1808) (as *Cirriformia tentaculata* (Montagu,1808))		+	
Monticellina dorsobranchialis (Kirkegaard, 1959)	+		
Opheliidae			
Armandia agilis (Andrews, 1891)	+		
Armandia cirrhosa Filippi, 1861		+	
Armandia maculata (Webster, 1884)	+	+	
Armandia bioculata Hartman, 1938		+	
Orbiniidae			
Leitoscoloplos foliosus (Hartman, 1951) (as *Haloscoloplos foliosus* (Hartman, 1951))	+		+
Leitoscoloplos fragilis (Verrill, 1873) (as *Haloscoloplos fragilis* (Verrill, 1873))		+	+
Leodamas rubra (Webster, 1879) (as *Scoloplos (Leodamas) rubra* (Webster, 1879))	+	+	
Nainereis sp.			+
Naineris setosa (Verril, 1900)		+	+
Protoaricia oerstedii (Claparède, 1864)		+	
Scoloplos robustus Rullier, 1964 (as *Leitoscoloplos robustus* (Verrill, 1873))	+	+	
Scoloplos texana (Maciolek and Holland, 1978)	+		
Scoloplos treadwelli Eising, 1914	+	+	+
Owenidae			
Galathowenia oculata (Zach, 1923)	+		
Owenia fusiformis Delle Chiaje, 1844		+	
Owenia sp.	+		
Paraonidae			
Aricidea (Acmira) hirsuta Arriaga-Hernández, Hernández-Alcántara and Solís-Weiss, 2013	+		
Aricidea (Strelzovia) suecica Eliason, 1920 (as *Aricidea suecica* Eliason, 1920)		+	+
Cirrophorus armatus (Glèmarec, 1966)		+	
Paraonides lyra (Southern, 1914) = *Paradoneis carmelitensis* Arriaga-Hernández, Hernández-Alcántara and Solís-Weiss, 2013	+		+
Pectinariidae			
Pectinaria meredithi Long, 1973	+		

Family - Species	**Habitat**		
	SB	SG	M
Moticellina sp.	+		
Aphelochaeta marioni (Saint-Joseph, 1894) (as *Tharyx marioni* (Saint-Joseph, 1894))			+
Aphelochaeta parva (Berkeley, 1929) (as *Tharyx parvus* (Berkeley, 1929))		+	+
Cossuridae			
Cossura delta Reish, 1958	+		+
Dorvilleidae			
Dorvillea rubra (Grube, 1856)	+		
Schistomeringos rudolphii(Delle-Chiaje, 1828)	+	+	+
Eunicidae			
*Lysidice ninetta*Audouin and Milne-Edwards, 1833		+	
Lysidice unicornis (Grube, 1840) (as *Nematonereis unicornis* (Grube, 1840)	+		
Marphysa aransensis Treadwell, 1939	+		
Marphysa sanguinea (Montagu, 1815)	+	+	+
Flabelligeridae			
Piromis eruca (Claparède, 1869) (as *Pherusa eruca* (Claparède, 1869))		+	
Piromis roberti (Hartman, 1951)	+		
Glyceridae			
Hemipodia sp. 1	+		
Goniadidae			
Glycinde multidens Müller, 1858 (as *Glycinde solitaria* (Webster, 1879))	+		+
Goniada echinulata Grube, 1870	+		
Goniada maculata Oersted, 1843	+		
Goniadides carolinae Day, 1973	+		
Ophiogoniada sp. 1	+		
Hesionidae			
Gryptis arenicola glabra (Hartman, 1961)		+	
Podarkeopsis brevipalpa (Hartmann-Schröder, 1959) (as *Gyptis brevipalpa* (Hartman-Schroeder, 1959))	+	+	
Pectinaria sp.	+		
Petta pellucida (Ehlers, 1887) (as *Petta pusilla* Malmgren, 1866)		+	
Petta tenuis Caullery, 1944			+
Petta sp.		+	
Phyllodocidae			
Hypereteone heteropoda Hartman, 1951 (as *Eteone heteropoda* Hartman, 1951)	+		
Hypereteone foliosa (Quatrefages, 1865) (as *Eteone foliosa* Quatrefages, 1866)	+		
Hypereteone lactea Claparède, 1868 (as *Eteone lactea* Claparède, 1868)	+		
Hypereteone sp. (as *Eteone* sp.)		+	
Phyllodoce arenae Webster, 1879	+		
Pilargidae			
Ancistrosyllis commensalis Gardiner, 1976	+		
Hermundura fauveli (Berkeley and Berkeley, 1941) (as *Loandalia fauveli* (Berkeley and Berkeley, 1941))	+	+	
Hermundura vivianneae (Salazar-Vallejo and Reyes-Berragán, 1990) (as *Parandalia vivianneae* Salazar-Vallejo and Reyes-Berragán, 1990)		+	
Hermundura sp. 1 (as *Parandalia* sp.)	+		+
Sigambra bassi (Hartman, 1945)		+	+
Sigambra grubii (Müller, 1858)	+		
Sigambra wassi Pettibone, 1966	+		
Polynoidae			
Antinoe microps Kinberg, 1856	+		
Antinoe uschakovi (Ibarzabal, 1988)	+		
Antinoe sp. 1	+		
Lepidonotus lacteus (Ehlers, 1887)	+		
Lepidonotus sublevis Verrill, 1873	+		
Malmgreniella taylori Pettibone, 1993	+		
Malmgreniella variegata (Treadwell, 1917)	+		
Malmgreniella sp. 1	+		

Family - Species	**Habitat**		
	SB	SG	M
Hesiocaeca sp.	+		
Oxydromus sp.	+		
Lumbrineridae			
Lumbrineris impatiens Claparède, 1868			+
Ninoe sp.	+		
Scoletoma candida (Treadwell, 1921)	+		
Scoletoma elongata (Treadwell, 1931)	+		
Scoletoma ernesti (Perkins, 1979)	+		
Scoletoma tenuis (Verrill, 1873) (as *Lumbrineris tenius* (Verrill, 1873))	+		
Scoletoma treadwelli (Hartman, 1956)	+		
Scoletoma verrilli (Perkins, 1979)	+		
Scoletoma sp.	+		
Maldanidae			
Sabaco elongatus (Verrill, 1873) (as *Branchioasychis americana* (Hartman, 1945))	+		+
Axiothella sp.			+
Axiothella mucosa(Andrews, 1891)		+	
Clymenella torquata (Leidy, 1855)	+		
Clymenella sp. 1	+		
Clymenura sp. 1	+		
Isocirrus sp. 1	+		
Maldane sp. 1	+		
Nereididae			
Allitta succinea (Frey and Leuckart, 1847) (as *Neanthes succinea* (Frey and Leuckhart, 1847))	+	+	+
Ceratonereis costae (Grube, 1840)		+	
Ceratonereis irritabilis (Webster, 1879)	+		
Ceratonereis versipedata (Ehlers, 1887)		+	
Ceratonereis sp.		+	
Dendronereis sp.		+	
Laeonereis culveri (Webster, 1879)			+
Laeonereis sp.	+		
Leonnates sp. 1	+		

Family - Species	**Habitat**		
	SB	SG	M
Malmgreniella sp. 2	+		
Sabellidae			
Branchioma sp.		+	
Megalomma bioculatum (Ehlers, 1887)	+		
Parasabella lacunosa (Perkins, 1984)	+		
Demonax microphthalmus (Verrill, 1873) (as *Sabella microphthalma* (Verrill, 1873))		+	
Sabella sp.		+	
Serpulidae			
Hydriodes parvus (Tradwell, 1902)		+	
Hydroides dianthus (Verrill, 1873)	+		
Hydroides protulicola Benedict, 1887	+		
Sigalionidae			
Sthenelais boa (Johnston, 1833)		+	
Sthenelais helenae Kinberg, 1856		+	
Sthenelais sp.	+		
Sthenolepis sp.	+		
Spionidae			
Dipolydora socialis (Schmarda, 1861) (as *Polydora socialis* (Schmarda, 1861))		+	
Dipolydora sp.	+		
Malacoceros vanderhorsti (Augener, 1927)			+
Minuspio ca. cirrifera Wirén, 1883	+		
Paraprionospio alata (Moore, 1923) (as *Prionospio (Paraprionospio) pinnata* Ehlers, 1901 or *Paraprionospio pinnata* (Ehlers, 1901))	+		+
Polydora cornuta Bosc, 1802 (as *Polydora ligni* (Webster, 1879))		+	+
*Polydora plena*Berkeley and Berkeley, 1936		+	
Prionospio ehlersi Fauvel, 1928		+	
*Prionospio heterobranquia*Moore, 1907	+	+	+
Prionospio pygmaeus Hartman, 1961	+		
Prionospio sp.		+	
Scolelepis ca. lighti Delgado-Blas, 2006	+		
Scolelepis squamata (O.F. Muller, 1806)	+	+	+
Spiophanes sp.		+	

Family - Species	**Habitat**		
	SB	SG	M
Leptonereis sp.			+
Neanthes acuminata Ehlers, 1868	+		
Neanthes caudata (Delle-Chiaje, 1827)		+	+
Nereis falsa Quatrefages, 1866		+	
Nereis grayi (Pettibone, 1956)		+	
Nereis micromma Harper, 1979	+		
Nereis oligohalina (Rioja, 1946)		+	
Nereis pelagica Linnaeus, 1758	+		
Nereis riisei Grube, 1857	+		
Nicon sp.	+		
Platynereis sp.			+
Stenoninereis martini Wesenberg-Lund, 1958			+
Oenonidae			
Arabella iricolor (Montangu, 1804)		+	+
Arabella sp.	+		
Drilonereis longa Webster, 1879	+		
Onuphidae			
Americonuphis magna(Andrews, 1891)		+	
Diopatra cuprea (Bosc, 1802)	+	+	+
Kinbergonuphis cedroensis (Fauchald, 1968)	+		
Kinbergonuphis pulchra (Fauchald, 1980)	+		
*Kinbergonuphis rubrescens (*Augener, 1906)	+		
Kinbergonuphis simoni (Santos, Day and Rice, 1981)	+		
Kinbergonuphis vermillionensis (Fauchald, 1968)	+		
Kinbergonuphis sp.	+		
Kinbergonuphis sp. 1	+		
Kinbergonuphis sp. 2	+		
Kinbergonuphis sp. 3	+		
Onuphis eremita (Audowin and Milne-Edward, 1833)		+	

Family - Species	**Habitat**		
	SB	SG	M
Streblospio benedicti (Webster, 1879)	+		+
Sternaspidae			
Sternaspis sp. 1	+		
Sternaspis sp. 2	+		
Syllidae			
Exogone dispar (Webster, 1879)	+		
Exogone lourei Berkeley and Berkeley, 1938	+		
Haplosyllis spongicola (Grube, 1855) (as *Syllis spongicola* (Grube, 1855))		+	
Perkinsyllis spinisetosa (San Martín, 1990)	+		
Pionosyllis sp.	+		
Prosphaerosyllis riseri (Perkins, 1980)	+		
Streptosyllis sp. 1	+		
Syllis garciai (Campoy, 1932)	+		
Syllis gracilis Grube, 1840	+		
Syllis mexicana (Rioja, 1960) (as *Elhersia mexicana* (Rioja, 1960))		+	+
*Sylliis variegata*Grube, 1860		+	
Syllis sp. (as *Syllis (Typosyllis)* sp.)		+	
Syllis hyalina Grube, 1863		+	
Syllis lagunae Tovar-Hernández, Hernández-Alcántara and Solís-Weiss, 2008		+	+
Terebellidae			
Loimia viridis Moore, 1903		+	
Polycirrus ca. haematodes (Claparède, 1864)	+		
Scionides sp.		+	
Terebella lapidaria Linnaeus, 1767		+	+
Terebella sp.		+	
Trichobranchidae			
Terebellides carmenensis Solís-Weiss, Fauchald and Blankensteyn, 1991 (as *Terebellides stroemi* Sars, 1835)	+		+
Terebellides lanai Solís-Weiss, Fauchald and Blankensteyn, 1991	+		

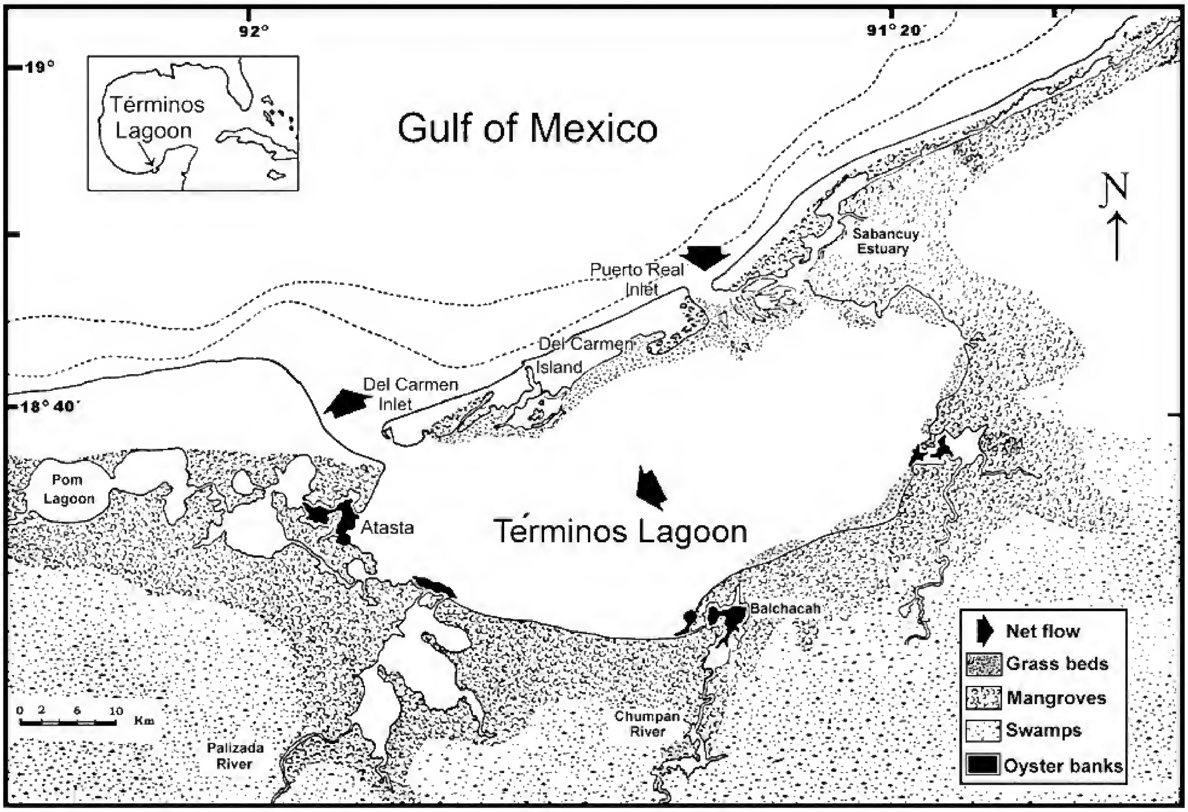

Figure 1. Location and distribution of habitats in Términos Lagoon, southern Gulf of México.

Results

For this study, 3,398 specimens (33 families and 119 species) were collected and identified in the soft bottoms of Términos Lagoon. Combining these results with the published information in its seagrasses and mangroves, we found that, so far, 190 species from 34 families have been recorded there (Table 1). The most diverse families were Nereididae (20 spp.), Spionidae (15 spp.) and Syllidae (14 spp.), although we note that their presence in the different habitats under study is highly variable. On the other hand, 68% of the families collected for this study were represented by only five or fewer species (fig. 2).

The distribution of the polychaete fauna in the Lagoon shows that soft bottoms constitute the more diversified habitat (119 species in 33 families), followed by the seagrasses with 75 species in 26 families, while the mangroves' environment has the least diverse fauna, with 42 species in 21 families. Although some caution is advisable when comparing the number of species of soft bottoms with literature records, mainly because the methodology and sampling effort are different, we observed that the faunal differences between habitats are more pronounced among families with the highest number of species: in the soft bottoms, six families are represented by eight species or more, but in the seagrasses only the nereidids and spionids (eight species) were similarly represented, and in the mangroves only these same families (Nereidae and Spionidae) were found with a maximum of six species (fig. 2).

In the soft bottoms, the highest number of species was found in the families Onuphidae (10 spp.), Nereididae and Polynoidae (both with 9 species), and Lumbrineridae, Syllidae and Spionidae (8 species). Although the nereidids and spionids were also diversified taxa in the seagrasses (both with 8 species) and mangroves (both with 6 species), the high diversity of onuphids, lumbrinerids and polynoids seems to be exclusive of the soft bottoms (fig. 2). Besides the polynoids, the families Acoetidae, Glyceridae and Sternaspidae have been only recorded in soft bottoms, while the Amphinomidae is the only one which has not been collected in that habitat. The family Syllidae occurs preferably in soft bottoms and seagrass beds, while the Maldanidae and Cossuridae are mainly found in soft bottoms and mangroves.

The distribution of the polychaetes in the three habitats shows that most species are not widely distributed: most of them, 154 species (81% of the total species), have been recorded in only one habitat, and only 5% of the polychaetes (10 species) are able to spread out to the different habitats of

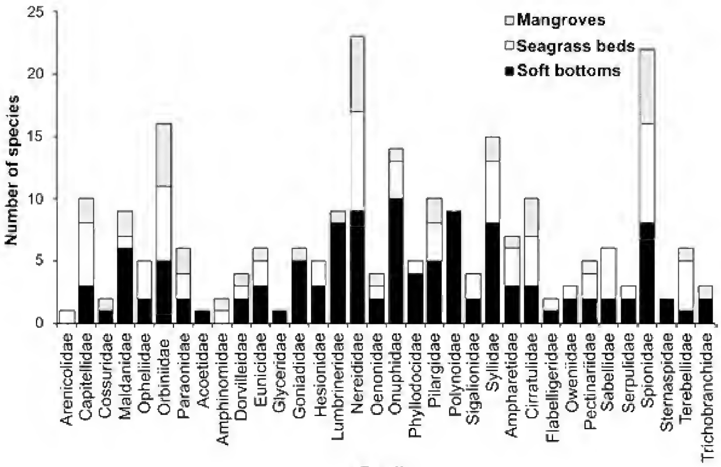

Figure 2. Number of species by family at each habitat in Términos Lagoon.

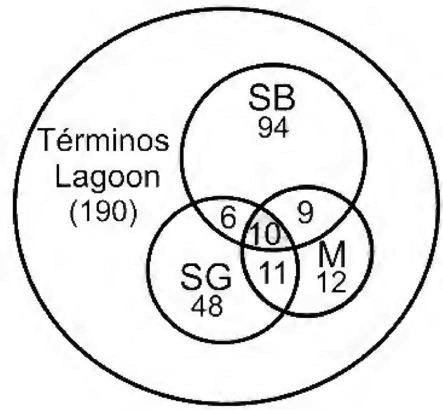

Figure 3. Distribution of the number of species by habitat in Términos Lagoon. (SB: soft bottoms; SG: seagrass beds; M: mangroves).

the lagoon (Table 1). Most of the species (7) distributed in all habitats have limited motility, and belong mainly either to Spionidae (*Scoloplos treadwelli* (Eising, 1941), *Prionospio heterobranchia* Moore, 1907 and *Scolelepis squamata* (O.F. Müller, 1806) or Capitellidae (*Capitella* sp. and *Mediomastus californiensis* (Hartman, 1944)). On the other hand, 26 species were found in two of the habitats, but the number of motile species clearly increased and more than 40% (11 species) can be classified as motile polychaetes (Table 1, fig. 3): six species are common to soft bottoms and seagrasses, nine species occur simultaneously in soft bottoms and mangroves, and 11 species are found in seagrasses and mangroves.

The biotic heterogeneity in the habitats of Términos Lagoon, evaluated by the ANOSIM test, shows that the global value of $R = 0.51$ is clearly much larger than any of the 999 permuted values ($p = 0.1\%$), rejecting the null hypothesis that there are no differences in the polychaetes' composition of the three environments. However, these faunal differences are not equal in each combination of groups, since pairwise tests show that the main separation of habitats, based on their species composition, is between the soft bottoms and seagrass beds ($R = 0.622$, $p < 0.1\%$). On the other hand, the polychaete species in the soft bottoms and mangroves ($R = 0.3$, $p = 1.1\%$), and in the seagrasses and mangrove environments ($R = 0.247$, $p = 5.5\%$) are very similar, and their faunal differences are not significant.

Discussion

The benthic macrofauna of tropical estuaries is commonly dominated by the Polychaetous annelids (Flint and Younk, 1983; Hernández-Alcántara and Solís-Weiss, 1995; Silva et al., 2011). This is due, among other things, to their highly diverse ethological habits which help them adapt to the (also) high environmental variability (Magalhaes and Barros, 2011). In this context, polychaetes are known for their tolerance of drastic environmental changes that make it possible for them to be well represented in lagoon-estuarine ecosystems (Gambi et al, 1997; Dittman, 2000; Rosa Filho et al., 2005). The "species minimum" concept indicates that the variable environmental features present in brackish systems, tend to exclude species (Remane, 1934). As a whole, the 190 species recorded in Términos Lagoon do not seem to represent outstanding diversity levels; this is especially true if they are compared to the 854 species of polychaetes recorded in the sublittoral soft bottoms of the Gulf of Mexico (Fauchald et al., 2009) even if, admittedly, the last are living in the more stable environment of the continental shelf. On the other hand, the comparison of biodiversity with other estuarine systems in the southern Gulf of Mexico is difficult, because knowledge of the polychaetes is very limited : only one species is known to have been collected in each of three estuaries, and a maximum of 70 species have been recorded in the other seven estuaries in this region. However, even considering that comparisons with other such studies in the region are to be taken with caution, due to the different sampling procedures used (Sicinski and Janowska, 1993; Gambi et al, 1997), the 190 species of polychaetes recorded in Términos Lagoon clearly represent a much higher biodiversity than that observed in many of the tropical estuarine systems of the American continent. Such is the case with the 83 taxa registered in an estuary of the Amazon (Silva et al., 2011), the 58 species of polychaetes recorded in an estuarine system in southern Brazil (Magalhaes and Barros, 2011), the 77 species registered in an impacted estuary of Rio de Janeiro, also in Brazil (Santi and Tavares, 2009), or the 120 species of polychaetes collected in an estuary in Costa Rica (Maurer and Vargas, 1984). Notwithstanding the relatively high diversity observed in Términos Lagoon, few families are diverse and widely distributed: of the 34 families recorded, 23 are represented by fewer than five species.

It is known that, along an estuary, the benthic communities vary widely in composition and are often associated with changes in salinity and type of sediment; in addition, the

greater complexity of habitats, such as the presence of vegetation or heterogeneous substrates, could be accompanied by increased species richness (Castel et al., 1989; Junoy and Vieitez, 1990). In this sense, we noted that few species (5%) occur in the whole lagoon and therefore, the faunal composition is different from one habitat to the next. However, these differences are only significant between the soft bottoms and seagrass environments. Most of these widely distributed species are deposit feeders or detritivores, which have already been reported as dominant in the soft bottoms of the lagoon (Hernández-Alcántara, 1991). For their part, the motile species, like *Marphysa sanguinea* (Montagu, 1815), *Alitta succinea* (Frey and Leuckart, 1847) and *Diopatra cuprea* (Bosc, 1802) have been usually reported on seagrass beds (Ibañez-Aguirre, 1986; Cruz-Ábrego et al., 1994).

It is necessary to have a much better knowledge of the life histories and behavior of the benthic fauna in the estuarine systems to achieve an adequate analysis of the structure of the marine communities. However, the complexity of the habitat structure created by the aquatic vegetation is an important factor in determining the diversity and composition of the communities, since they provide feeding resources and refuges to many invertebrates. This, in turn, generates differences with the fauna in unstructured habitats, such as soft bottoms (Minello et al., 2003).

In the mangrove sediments, the number of species was the lowest and the polychaete species present were very similar to those recorded in soft bottoms or seagrasses. In this case, the sampling effort was lower than in the other habitats, which could lead to an underestimate of their real biodiversity. However, few species are limited to the mangroves and, for many this environment is an extension of their "normal" habitat (Hutchings and Recher, 1982; Hernández-Alcántara and Solís-Weiss, 1991). Anyhow, it is possible that in this lagoon, an active faunal exchange takes place between the mangroves and the other two habitats studied (Hernández-Alcántara and Solís-Weiss, 1991).

Differences in the structure of the habitats analyzed and the highly variable environmental changes, which characterize the lagoon-estuarine systems, determine the high number of species recorded exclusively in one habitat (154 species), but it may also provide a particular space for opportunistic species, like, in this instance, the spionid polychaetes. Spionids are one of the most diverse families in the lagoon and their characteristic species *Scoloplos treadwelli*, *Prionospio heterobranchia* and *Scolelepis squamata*, occur in the three habitats while *Paraprionospio alata* (Moore, 1923), *Streblospio benedicti* (Webster, 1879) and *Polydora cornuta* Bosc, 1802, have been collected in two habitats at the same time. Spionids are often abundant in fine sediments and they can show marked population fluctuations. Many are opportunistic, responding to enrichment and disturbance (Pearson and Rosenberg, 1978). Another species frequently recorded in the three habitats of the Términos Lagoon, *Capitella* sp. (Capitellidae), is closely related to *Capitella capitata* (Fabricius, 1780), which thrives in organic-rich environments and has been used as a biological indicator of organic pollution (Reish, 1959). The enrichment of the sediments in this lagoon could well cause the presence of these opportunistic polychaetes. However, this "species" is actually considered to include a group of unnamed sibling species with different life histories and reproductive attributes, but with only slight morphological differences between them (Grassle and Grassle, 1976). That is why, in this study, the analyzed individuals were left as *Capitella* sp., and the previous records of *C. capitata* in the lagoon, whose specimens were also revised, were all renamed *Capitella* sp., until their taxonomic status can be elucidated.

The presence of root structures in seagrass beds may reduce the water flow, increase the content of organic matter in that sediment and provide refuge from predation for benthic invertebrates (Orth et al., 1984), encouraging the presence of a rich benthic fauna. However, in Términos Lagoon, the number of species of polychaetes is clearly higher in soft bottoms than in seagrasses, which is probably the result of this being the largest habitat of the lagoon, but also of its large variation in environmental conditions, while the seagrass beds are mainly distributed in areas with strong marine influence from southern Del Carmen Island and patches of different size at the eastern end of the lagoon (both sides of Puerto Real inlet).

The faunal composition is related to habitat type in estuarine environments, and usually the number of species is higher in a structured habitat, such as the seagrasses, compared to soft bottoms devoid of vegetation (Ferraro and Cole, 2004; Hosack et al., 2006). However, the results obtained in this study do not support previous observations which suggest that complex habitat structure increases the presence of species (Hosack et al., 2006), and the faunal differences could be associated to other physical-chemical factors. Unfortunately, the scarce information available on the structural organization of the benthic communities, not only in Términos Lagoon, but in all the tropical estuarine systems of the Gulf of Mexico, makes it difficult to evaluate and verify these statements. Besides, from the 1980s, Términos Lagoon conditions have been changing constantly, with an increase of marine influence, more turbidity, a general decrease of depth, and even a decrease in the seagrass meadows, particularly around Ciudad del Carmen (the island city), associated with an increasingly fast urbanization (Villéger et al., 2010). This could modify the relationships between the species already settled, new settlements and their distributional patterns.

Anyway, the information about the biodiversity of the polychaetes from this study is important as a departure point for understanding the ecological mechanisms prevalent in this lagoon-estuarine system, since the polychaetes have been widely used as indicators of the general "health" in benthic communities, especially those under pollution impacts (Dean, 2008). Unfortunately, anthropogenic influence is increasing in those systems all along the southern region of the Gulf of Mexico, negatively affecting them. In 1971, the largest deposit of hydrocarbons in Mexico (Cantarell) and one of the largest in the world was discovered, precisely in front of Términos Lagoon on the continental shelf. Following this discovery the area became one of the most important economic zones of the country, but this development triggered the transformation of previously rural areas into urban zones quite rapidly (Soto-Galera et al., 2010).

This exceptional economic development and its many related activities (oil extraction and associated industry, fisheries, tourism, fast urbanization, etc.) has increased the exploitation and deterioration of the natural resources of the region, and its traditional activities which often collide with the modern and excessively fast development (Sánchez-Gil et al., 2004).

Finally, it is necessary to emphasize the fact that benthic communities are severely threatened by the worsening conditions of those habitats due to human activities (Snelgrove et al., 1997), and that only a small fraction of the species which live in the benthos in general have been described in tropical regions. So, there is a high probability that many of them will disappear even before they are known (Snelgrove, 1998, 1999), in particular, in the southern Gulf of Mexico, where oil extraction and processing of its derivates take place in the vicinity of these lagoon-estuarine systems. Even if the characterization of the polychaete fauna found in Términos Lagoon is still incomplete, the information generated by this type of biotic inventory, including spatial and seasonal variation, is key to understanding the functioning of these communities and will hopefully further stimulate the study of these environments in the southern region of the Gulf of Mexico. In turn, those studies will help to manage and protect these natural resources, while allowing the rational exploitation of the oil and fisheries industry.

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Description of a new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicidae) from the west coast of Peninsular Malaysia and comparisons with species from *Marphysa* Group A from the Indo-West Pacific and Indian Ocean

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Abstract

Idris, I., Hutchings, P.A. and Arshad, A. 2014. Description of a new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicidae) from the west coast of Peninsular Malaysia and comparisons with species from *Marphysa* Group A from the Indo-West Pacific and Indian Ocean. *Memoirs of Museum Victoria* 71: 109–121.

A new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicidae) is described from the west coast of Peninsular Malaysia and compared with species from *Marphysa* Group A from the Indo-West Pacific and Indian Ocean. The number of species known within *Marphysa* Group A has been increased, and the concept that *M. mossambica* is a widely distributed species in the Indo-Pacific is refuted. The new species is commercially important and occurs in the mangrove forest along the west coast of Peninsular Malaysia. Notes on the biology, ecology and commercial aspects of *M. moribidii* sp. nov. are presented.

Keywords polychaete, mangrove, intertidal, commercial polychaete, bait worm, *Marphysa*

Introduction

Studies on polychaete taxonomy in Malaysia are relatively few compared with those of neighbouring countries (Paxton and Chou, 2000; Aungtonya et al., 2002; Al-Hakim and Glasby, 2004; Chan, 2009; Rajasekaran and Fernando, 2012). Publications on polychaetes are scattered, with no specific taxon targeted; examples of the publications are Ong (1995) and Nishi (2001). A recent literature review by Idris and Arshad (2013) indicates that 64 species from 31 families of polychaete have been identified in Malaysia since 1866. Nevertheless, with a total 4675 km of Malaysian coastline it is suggested that the number of polychaete species recorded will increase with additional studies.

As part of the effort to increase the number of identified polychaete species in Peninsular Malaysia, a survey was conducted to identify polychaetes used as baitworms. This survey identified seven species (from four families) that are harvested and used regularly by local recreational fishers (Idris et al., 2012; Idris and Arshad, 2013). One of the species reported was identified as *Marphysa* cf. *mossambica* (Peters, 1854) and it was found along the west coast of Peninsular Malaysia within the mangrove forest.

The species *Marphysa mossambica* was initially described as *Eunice mossambica* by Peters (1854) with reference to specimens collected from Mozambique:

"*E. mossambica* sp., closely related to *E. sanguinea* Montagu, but different concerning the position of the antennae and the position of the eyes. The external antennae do not protrude beyond the posterior head region and the eyes are located at the outer part of the basis of the internal antennae. Distributed in sandy coastal regions, from Mossambique to Mossimboa, from 11° to 15° south" (sic).

Unfortunately, Peters (1854) description on the species is too brief and did not mention chaetal types or dentition of the jaws, which are critical characters for the identification of this group of worms. Over 100 years later, Fauchald (1987) examined the lectotype material deposited in the Zoologische

Museum, Berlin, Germany, (ZMB F2046) and provided a detailed description of the species.

In 1865, Kinberg identified a specimen from Sydney Harbour (Port Jackson), Australia, as *Nauphanta novaehollandiae* Kinberg, 1865. Many polychaete workers, namely Gravier (1900), Crossland (1903) and Augener (1922), agreed that *N. novaehollandiae* should be synonymised with *M. mossambica*. Augener (1922), however, appears to have preferred to use the name *N. novaehollandiae* rather than *M. mossambica*, as the description made by Kinberg (1865) is more complete. Fauchald (1987), after examining the lectotype (ZMB F2046) and paralectotypes (ZMB 47, ZMB 4005), transferred *M. mossambica* into the genus *Nauphanta* as 'fan-shaped chaetae' were present and there was a total absence of compound chaetae. This differs from Fauchald (1970), who proposed that species of *Marphysa* could be split into five groups, with one referred to as Group A, characterised by lacking any composite chaetae; *M. mossambica* is included within this group. A recent study by Glasby and Hutchings (2010) has suggested that the synonymy of *N. novaehollandiae* with *M. mossambica* be reinstated. They suggested that while Fauchald (1987) regarded the differences where the branchiae and hooks begin as species specific, in fact these differences can be due to size-related variation. A more recent review of the family Eunicidae by Zanol et al. (2014) has included a new character for *M. mossambica*—a wide pectinate chaeta with wide teeth on posterior chaetigers.

Until 2010, *Marphysa* Group A, comprised only two species—*M. mossambica* and *M. simplex* Treadwell, 1922. The latter species was then synonymised with *M. mossambica* (Glasby and Hutchings, 2010), leaving *M. mossambica* as the only species in this group. Nevertheless, a detailed examination of *Marphysa* cf. *mossambica* specimens from Peninsular Malaysia found consistent morphological differences compared with the description of *M. mossambica* that warrant the description of a new species in *Marphysa* Group A.

Materials and methods

Specimens were collected from various locations on the west coast of Peninsular Malaysia (fig. 1). Malaysia is located in the central part of south-east Asia and consists of two land masses — Peninsular Malaysia and east Malaysia. Peninsular Malaysia is a land bordered with Thailand in the north, while Indonesia and Singapore share the maritime limits in the west and south, respectively. The east Malaysia consist of two states i.e. Sabah and Sarawak. Both states are located on the northern part of Borneo island, sharing land border with Indonesia in the south, while Philippines share the maritime border at the east of Sabah. A sovereign Brunei is located on the upper part of the state border between Sabah and Sarawak.

All sampling locations on the west coast of Peninsular Malaysia are similar in terms of habitat — mangrove forest and mudflats. Specimens were relaxed in 7% MgCl, then fixed in 10% formalin and later preserved in 70% ethanol.

Material was examined using stereo (Olympus SZ) and compound (Nikon Eclipse E400) microscopes. Detail characters on parapodia 3, 10, 20, 50, 100, 150, 260, 400 and 456 were observed using the scanning electron microscope (SEM) Zeiss EVO LS15 SEM with a Robinson Backscatter Detector. Biometric measurements of the width of chaetiger 10 (including parapodia) and number of chaetigers on which the branchiae occurred from 35 specimens were made using a stereo microscope with a calibrated eye graticule. Analysis and graphs of size-related data were made using Microsoft Excel® 2010.

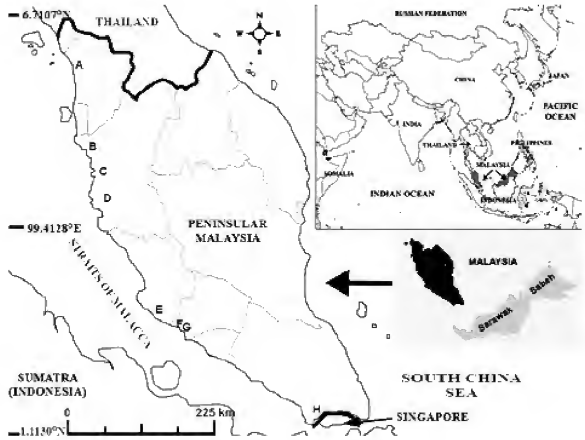

Figure 1. Locations of *Marphysa moribidii* sp. nov. in Peninsular Malaysia: A, Sg. Merbuk estuary, Kedah; B, Kuala Gula, Perak; C, Kg. Terubong Laut, Perak; D, Kg. Sitiawan, Perak; E, Morib mangrove, Selangor; F, Kuala Lukut, Negeri Sembilan; G, Bt. 4, Port Dickson, Negeri Sembilan; H, Tg, Kupang, Johor.

Ethanol-preserved specimens were deposited at the Australian Museum, Sydney, (AM) and at the Museum and Art Gallery of the Northern Territory (NTM), Australia.

Lectotype specimens (ZMB 4005, as stated in the specimen jar from the Zoologische Museum, Berlin, not ZMB F2046, as stated in Fauchald (1987)) were re-examined as well as a SEM stub with parapodia from various regions of the body.

Abbreviations

AM Australian Museum, Sydney
NTM Museum and Art Gallery of the Northern Territory, Australia
ZMB Zoologische Museum, Berlin, Germany

Systematics

Order **Eunicida** Dales, 1962

Family **Eunicidae** Berthold, 1827

Genus ***Marphysa*** Quatrefages, 1865

Marphysa moribidii Idris, Hutchings and Arshad sp. nov.

Zoobank LSID. http://zoobank.org/urn:lsid:zoobank.org:act:C693255A-0A15-4162-B9D2-B4EFAFD0C341

Figures 2, 3, 4

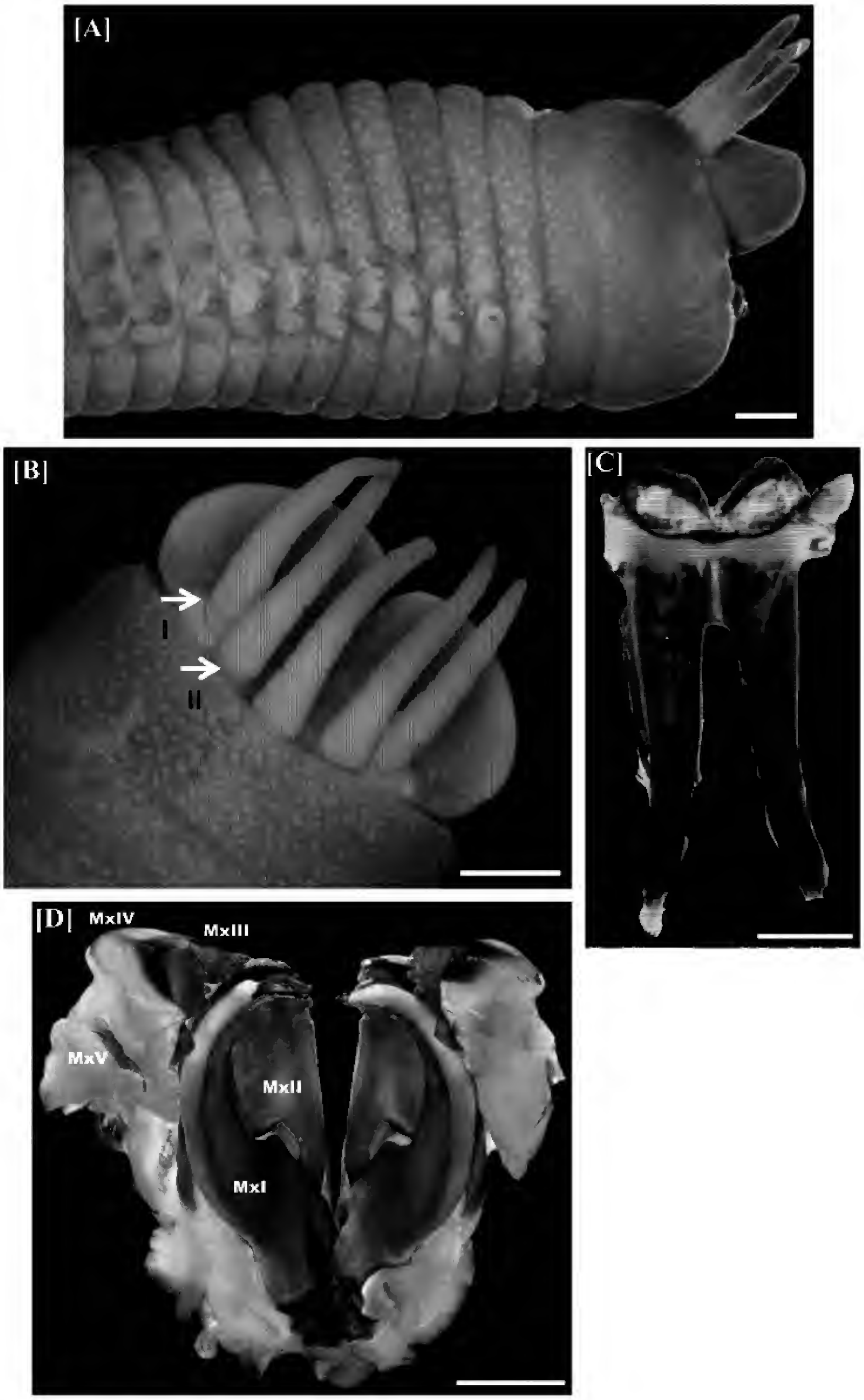

Figure 2. *Marphysa moribidii* sp. nov. [A] anterior section, lateral view. Note on the white spots on the epidermis of the specimen; [B], anterior section, dorsal view, showing the palpophore (I) and ceratophores (II); [C] mandible; [D] maxillae. [A, B, C] from non-type specimens; [D], from holotype (AM W43731). Mx = Maxillae. Scale bars: [A, B, C, D] = 1 mm.

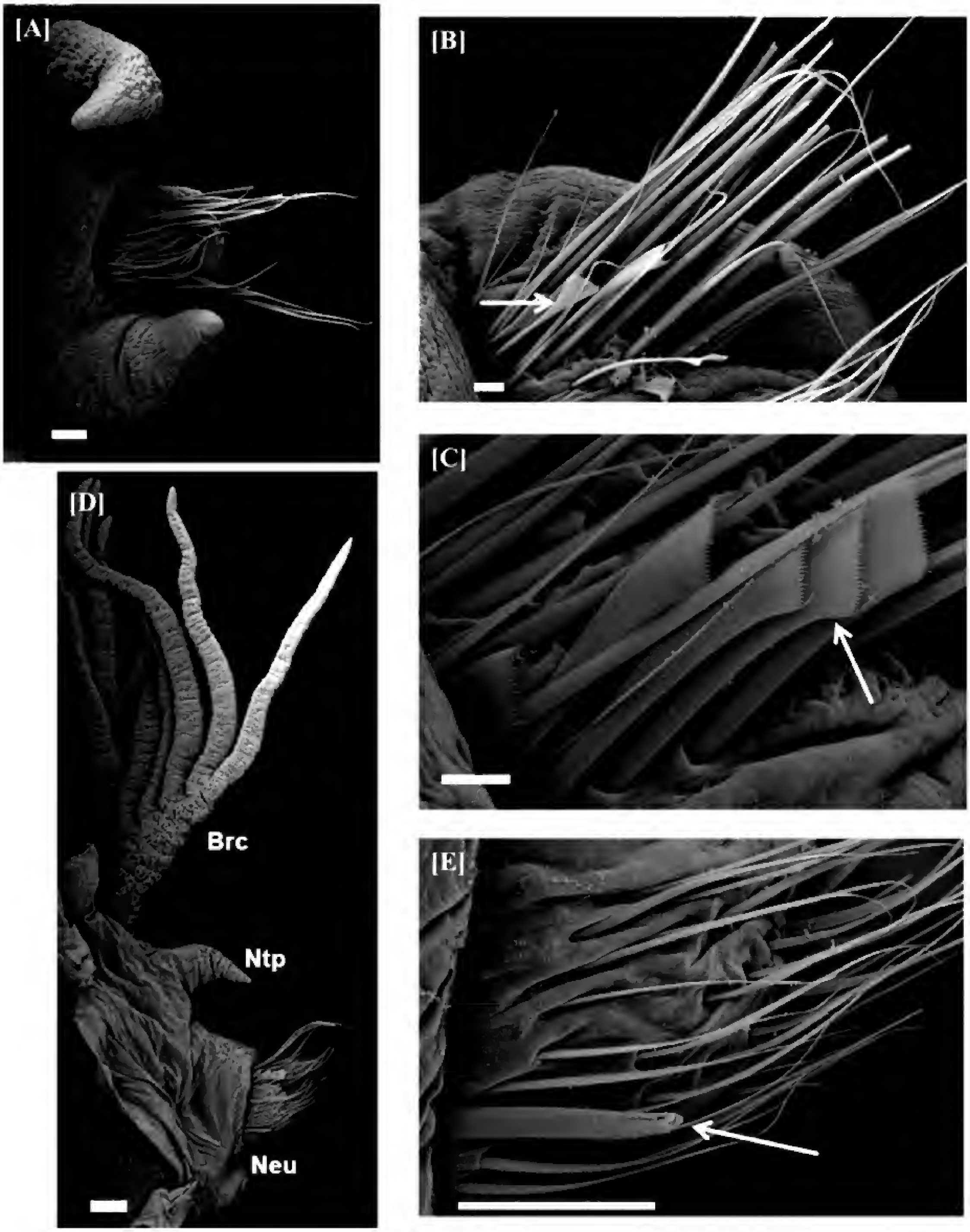

Figure 3. *Marphysa moribidiii* sp. nov., [A] limbate and simple capillary chaetae, chaetiger 3; [B] symmetrical pectinate chaeta on the supra-position (arrow), chaetiger 50; [C] asymmetrical pectinate chaetae (arrow), chaetiger 10; [D] whole parapodium with branchia, showing relative length with notopodial cirrus, chaetiger 150; [E] sub-acicular hook (arrow), chaetiger 150. Brc = Branchia; Ntp = Notopodial cirrus; Neu = Neuropodial cirrus. [A, C, D, E] = Paratype, AM W38692; [B] = Non-type (AM W38687). Scale bars: [A, D, E] = 100 μm; [B, C] = 20 μm.

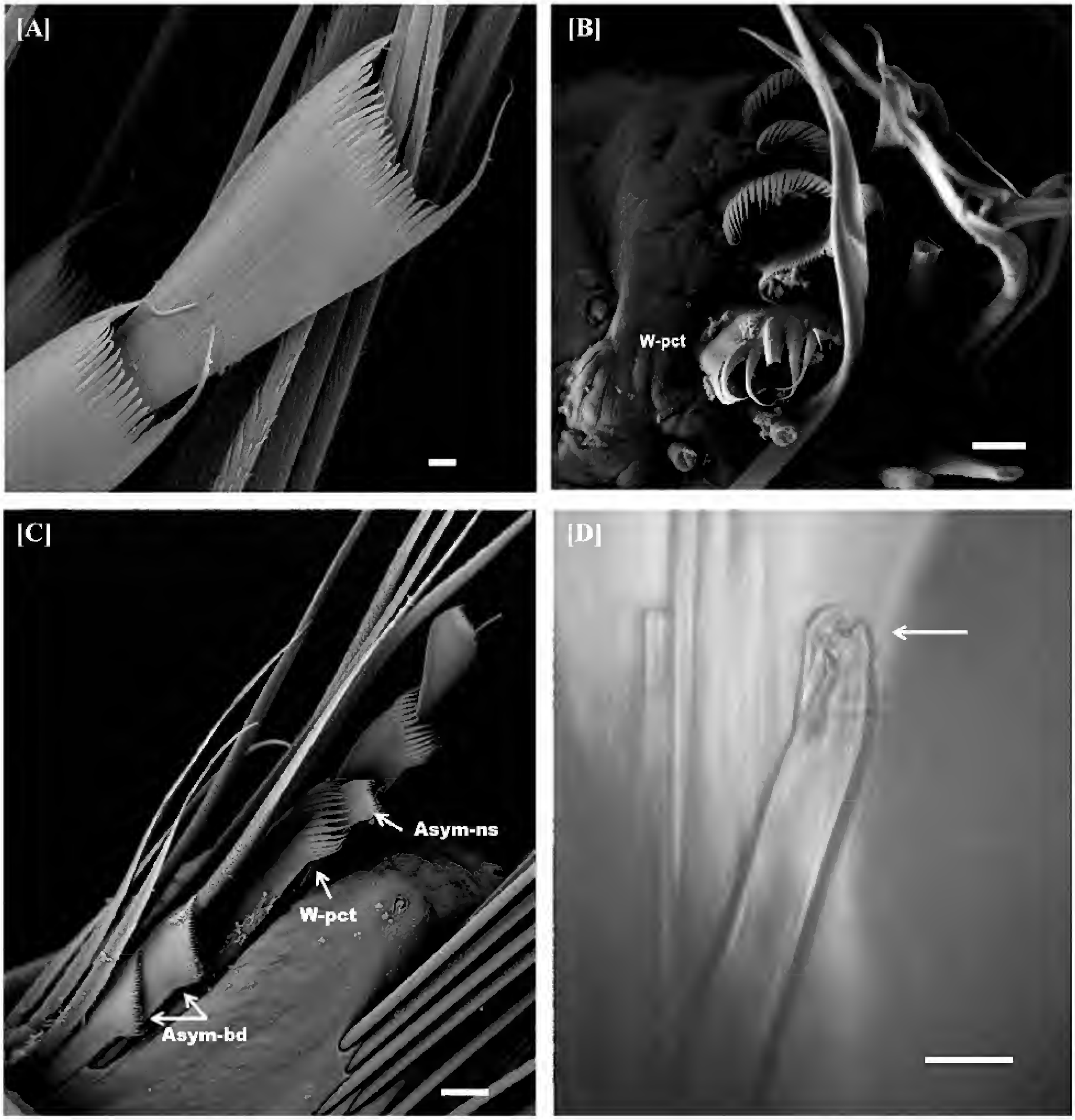

Figure 4. *Marphysa moribidii* sp. nov. [A] details of symmetrical pectinate chaetae, chaetiger 20; [B] wide pectinate chaeta with wide teeth (W-pct), dorsal view, chaetiger 400; [C] asymmetrical pectinate chaetae, all types, chaetiger 456; [D] (arrow) detail of bidentate sub-acicular hook, chaetiger 98. Asym-bd = asymmetrical pectinate with broad shaft; Asym-ns = asymmetrical pectinate with narrow shaft; [A] = non-type (AM W38687); [B, C] = holotype (AM W43731), [D] = non-type specimen. Scale bars: [A] = 3 μm; [B, C, D] = 20 μm.

Material examined. Holotype. AM W43731 – male, complete, Pantai Kelanang, Morib, Selangor, 2.75827°N 101.4379°E, coll. I. Idris 19 Jul 2012.

Paratypes. AM W38690 – 2 specs (1 male and 1 female), AM W38691 – 1 female, AM W38692 – 1 female, NTM W024777 – 1 spec. Data same as holotype.

Other material examined: AM W38684 – 1 female complete, NTM W024778 – 1 spec., Sg. Merbuk tributary, 5.6392°N 100.4138°E (range 10 km), coll. local bait digger 9 Feb 2011; AM W38685 – 2 females, NTM W024776 – 1 spec., Kg. Terubong Laut, Larut, Perak, 4.5659°N 100.6557°E (range 2 km), coll. local bait digger 8 Feb 2011; AM W38686 – 1 male, Kuala Gula, Perak, 4.9285°N 100.5086°'E (range 5 km), coll. local bait digger 11 Feb. 2011; AM W38689 – 1 male, Tg. Kupang (2nd link bridge), Johore, 1.3956°N 103.6221°E, coll. I. Idris 5 Nov 2010; AM W38693 – 2 males, Kg. Sitiawan, Lumut, Perak, 4.2498°N 100.6893°E, coll. local bait digger 8 Feb 2011; AM W38694 – 1 male, NTM W024775 – 1 spec., Bt. 4, Port Dickson, Negeri Sembilan, 2.5034°N 101.8352°E (range 2 km), coll. local bait digger 20 Jan 2011; AM W38695 – 1 female, NTM W024774 – 1 spec., Kuala Lukut, Negeri Sembilan, 2.5698°N 101.7945°E, coll. local bait digger 20 Jan 2011.

Comparative material examined. *Eunice mossambica* ZMB 4005 Lectotype – female, Mozambique, coll. and det. Peters 1854; *Marphysa mossambica* AM W35469 – female, Dumangas, Iloilo, Philippines, 10.7968°N 122.6695°E, coll. J. Monteros-Recente 7 May 2010, det. C.J. Glasby.

Measurement. Holotype. Mature male (with gametes visible through body wall in parapodia on anterior and mid body segments), complete specimen total length of 333 mm in preserved solution (70% ethanol). Body width at chaetiger 10 (with parapodia) 9.76 mm, total number of segments 465. Paratypes mostly incomplete, body width at chaetiger 10 (with parapodia) 4.8 – 8.0 mm. Longest preserved specimen is AM W38684, with total length of 612 mm and 780 segments.

Description. Holotype (paratype values in parentheses). Body long and slender. Cylindrical at anteriormost part of metastomium until chaetiger 7 (3 – 7) but gradually becoming flattened dorsoventrally towards posterior end. Live worm with blood red branchiae. Anterior metastomium dark red gradually became lighter, slightly transparent towards posterior allowing the alimentary canal to be seen. Preserved specimen olive green with white spots dorsoventrally distributed on anterior; continue mid-dorsally along metastomium to about one-quarter of the body length (figs. 2A and B). White spots visible on live specimen, but faint and not detected on some specimens if the worm was not completely cleaned of adhering sediment.

Prostomium consists of semi-circular, bilobed upper lips with distinct middle notch, appearing as if two lobes present (fig. 2B). Prostomium surface and appendages with almost smooth surface, without articulations. Prostomium appendages slightly curved. Median antenna about the same length as lateral antennae and slightly longer than palps (0.2 – 0.5 times longer). Antennae (median and lateral) about twice the length of the prostomium. Ceratophores and palpophores present, cylindrical, short, with no articulations (fig. 2B). No gap between palps and lateral antennae, but small gaps exist between median and lateral antennae. Eyes absent. Peristomium consists of two rings with length of first ring about 2.5 times longer than second ring. Dorsal part of first ring slightly longer than ventral side including peristomium fold. Lateral and ventral sides of first peristomium ring (lateral and lower lips) covered with abundant folds (fig. 2A). Mandibles dark brown but with white calcified layer on cutting plates (paratype: transparent cutting plates). Cutting plate sub-oval, flat, no dentition on cutting edge, slightly rough surface with carrier almost parallel (fig. 2C). Maxillae dark brown but becoming paler on edge (fig. 2D). Dental formulae: MxI = 1 + 1, MxII = 4 + 4 (4 + 5 – 6), MxIII = 6 + 0 (8 + 0), MxIV = 4 + 8 (7 + 8) and MxV = 1 + 1 (1 + 1). MxVI is absent.

Parapodia consisting of notopodial and neuropodial cirri, as well as post-chaetal lobe. Pre-chaetal lobe absent (fig. 3A). Notopodial cirri gradually change from subulate to conical towards posterior parapodia. Neuropodia initially with conical cirri gradually becoming sub-conical towards posterior end. Base of notopodial cirri sub-ovulate in anterior chaetigers without inflation but gradually becoming circular in median and posterior chaetigers. Post-chaetal lobe sub-conical in first chaetiger, gradually becoming sub-triangular by chaetiger three, low and broad from chaetiger four to around chaetiger 130, then gradually decreasing in size from chaetiger 131 towards posterior end. Branchiae first emerge from base of dorsal cirri at chaetiger 35 (33 – 39) and disappear by last 20 chaetigers. Number of branchial filaments gradually increases from one to maximum 11 (6 – 14), filaments arranged as pectinate type in mid-body, number of filaments decreases to one filament on posterior segments (fig. 3D). Length of branchial stem shorter than neuropodial cirri by chaetiger 35 (33 – 39), the chaetiger on which branchiae first emerge. Branchial stem length then gradually increases until about 10 – 15 times longer than notopodial cirri by chaetiger 70, where maximum number of branchial filaments is reached (13 in type specimens, 14 in non-types).

Chaetae divided into two fascicles: supra-acicular and sub-acicular chaetae with aciculae located in middle (lateral view) (fig. 3A). Six types of chaetae present: thick limbate; slender capillary; symmetrical pectinate; asymmetrical pectinate with narrow shaft; asymmetrical pectinate chaetae with broad shaft; and wide pectinate chaetae with wide teeth (figs. 3B, C; figs. 4A, B, C). Limbate chaetae longer and thicker than capillaries but both serrated. Limbate and capillary chaetae present in both fascicles throughout body. Number of limbate chaetae range from 28 – 41 until about chaetiger 100 and then reducing to 13 – 19 chaetae in posterior region. Capillary chaetae present in small numbers (<10) throughout. Symmetrical pectinate chaetae characterized as having both outer teeth of the same length with slender shaft (fig. 3B; fig. 4A). Symmetrical pectinate chaetae present from chaetiger five (chaetiger three in paratype), apparently absent after chaetiger five until chaetiger 50, then present again from chaetiger 51 onwards. Asymmetrical pectinate chaetae only present from chaetiger 100 onwards in type specimens and characterised as having the outer teeth of different length to the median teeth with broad or narrow shafts (fig. 3C, figs. 4B, C). The wide-toothed pectinate chaetae with wide body are present in holotype from about chaetiger 400 onwards (figs. 4B, C). Numbers of pectinate chaetae per parapodia ranged from one to six for both holotype and paratypes. Aciculae 3 –

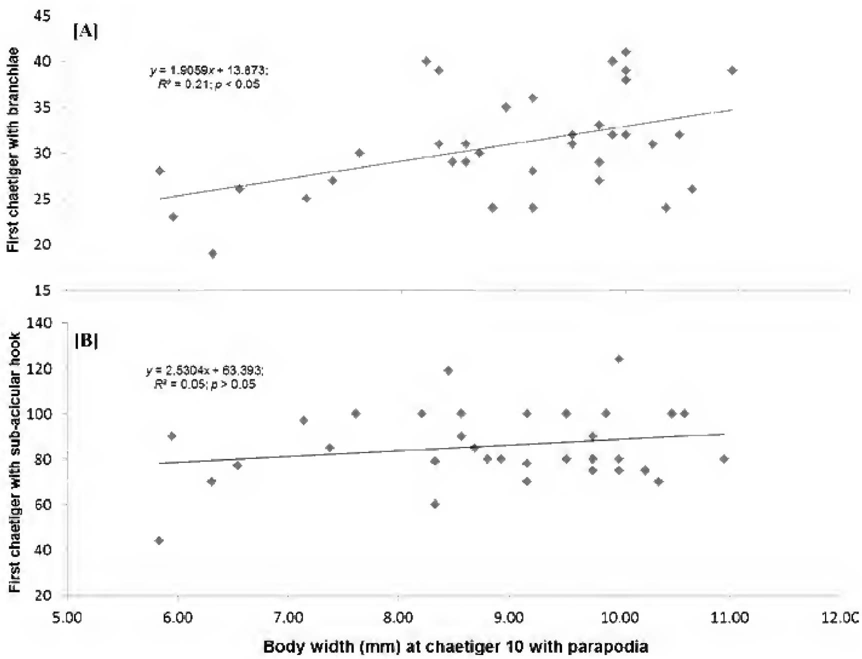

Figure 5. Relationships between body width (at chaetiger 10 with parapodia) and [A], first chaetiger with branchiae; and [B], first chaetiger with subacicular hook of *Marphysa moribidii* sp. nov., from Morib mangrove, Malaysia. Regression equations and coefficients are for all data points ($n = 35$).

4 per parapodium, dark brown, distally pointed, and arranged straight and almost parallel between fascicles. No sub-acicular hooks in holotype; however in paratypes, bidentate hooks present from median chaetiger (chaetiger 71 in one paratype only), but occurring irregularly.

Pygidium typical of *Marphysa* species, two pairs of unequally sized pygidial cirri inserted ventrally, arranged on top of each other. Largest: dorsal, two times height of pygidium, smallest about one-quarter height of pygidium.

Etymology. The name '*moribidii*' refers to the location (Morib mangrove) where the type specimens were collected. Morib is also the landing site of the 46th Indian Beach Group under the Allied Forces to mark the end of the Japanese occupation of Malaya in 1945. The local name for *Marphysa moribidii* is ruat bakau (mangrove worm).

Intraspecific variation. Information on the morphological variation present in this species is based upon detailed examination of 914 specimens collected from the type locality from June 2011 to December 2012. However, of these, only 136 specimens were complete. The large number of incomplete specimens was due to the method of collecting by digging with a shovel and the fragility of the animals. Length of complete specimens in preserved 70% ethanol ranges from 7 – 477 mm, with the number of chaetigers varying from 113 – 580. However, there is an incomplete specimen with 600 chaetigers, indicating that the number of chaetigers can be higher or similar to the longest deposited specimen (AM W38684). Body colour varies from dark olive green to light brown. In some specimens, the white spots are absent. Peristomium flap on the anterior of first ring can extend until it covers the ceratophores and palpophores. However, in some specimens, the flap was not detected or was reduced.

The chaetiger number at which the branchiae commence varies greatly in non-type specimens. The branchiae begin from chaetiger 4 – 63 as a single filament (in some specimens two to three filaments) and can reach a maximum number of 14 filaments in non-type specimens. The distribution of bidentate, sub-acicular hooks is irregular; they are present from chaetiger 44 in some specimens (figs. 3E, 4D). Some specimens also possess two bidentate sub-acicular hooks at the midsection of the body (chaetigers 60 – 78).

The relationship between body width at chaetiger 10 and the chaetiger on which the branchiae appear (fig. 5A) shows a significant positive linear relationship ($R^2 = 0.21$; $n = 35$; $p < 0.05$).

The positive relationship for these morphological characters is similar to that found in *M.* cf. *mossambica* and occurring in the synonymised *M. novaehollandiae* (Glasby and Hutchings, 2010). However, the correlation value of *M. moribidii* and *M.* cf. *mossambica* differs significantly between the two species ($z = 3.19$, $p < 0.05$).

Moreover, the relationship between body width and the chaetiger on which the sub-acicular hook appears (fig. 5B) is not statistically significant ($R^2 = 0.05$; $n = 35$; $p > 0.05$). Thus the appearance of sub-acicular hooks on the parapodia is not in a predictable pattern for *M. moribidii* sp.nov.

Table 1. Morphological comparison between *Marphysa moribidii* sp. nov., *Marphysa mossambica* and *Marphysa* cf. *mossambica* (*sensu* Fauchald, 1987 and *sensu* Glasby and Hutchings, 2010) from Australia. Variations (ranges) in population in parentheses. [a]Distinguishing characteristic.

Characteristics	***Marphysa moribidii* sp. nov. (present study)**	***Eunice (Marphysa) mossambica* (Lectotype; Fauchald 1987 and present study)**	***Nauphanta novaehollandiae (Marphysa cf. mossambica* (*sensu* Fauchald 1987))**	***Marphysa* cf. *mossambica* (*sensu* Glasby and Hutchings, 2010)**
Location	West coast of Peninsular Malaysia (type locality: Morib mangrove)	South-west of Indian Ocean (Mozambique)	South-west Pacific Ocean (Australia)	Arafura Sea, south-east Indian Ocean (Australia)
Preserved body length (mm) (chaetiger 10 incl. parapodium)	9.8 (7.1 – 47.7)	10	40 (measured at chaetiger 20)	(2.2 – 9.0)
Body shape	Rounded initially, but becoming flatter starting from chaetiger 7 towards posterior	Rounded until chaetiger 10, then flattened towards posterior	Not mentioned	Rounded initially, flattened in middle and posterior body
Body pigmentation[a]	Olive green with white spots on dorsal and ventral sides of anterior section	Not mentioned (no white spots, light brown pigmentation; pers. obs.)	Not mentioned	No pigmentation
Prostomium shape	Anteriorly truncate, bilobed with distinct mid notch	Frontally truncate, bilobed with shallow mid notch	Anteriorly truncate, bilobed with distinct mid notch	Bilobed
Prostomium appendages (surface)	Smooth throughout	Smooth throughout	Smooth throughout	Smooth throughout
Ceratophore	Present	Present	Present	Present
Median antenna (length relative to palps)	Slightly longer than palps	Mid antenna reaching to chaetiger 3	Slightly longer	Twice length of palps
Median antenna (length relative to prostomium)	About ~1 time (2 times) length of prostomium	1.5 times longer than prostomium	Reaching chaetiger 2	About 1.5 times length of prostomium
Mandibles	Flat, dark-brown carrier and calcerous layer on cutting plate	Not mentioned (light brown, transparent at the edge; pers. obs.)	Not mentioned	Dark brown; lighter-coloured cutting plate
Maxillae	Dark brown, but becoming lighter at the edge	Not mentioned	Not mentioned	Brown, edges and sutures darker brown
MxI (number of teeth; left + right)	1 + 1	1 + 1	1 + 1	
MxII (number of teeth; left + right)	4 + 4 (5 – 6)	(5 – 7) + (5 – 7)	5 + 6	(5 – 7)
MxIII (number of teeth; left + right)	6 (8) + 0	(4 – 7) + 0	? + 0	

Characteristics	***Marphysa moribidii* sp. nov. (present study)**	***Eunice (Marphysa) mossambica* (Lectotype; Fauchald 1987 and present study)**	***Nauphanta novaehollandiae (Marphysa cf. mossambica (sensu* Fauchald 1987))**	***Marphysa* cf. *mossambica* (*sensu* Glasby and Hutchings, 2010)**
MxIV (number of teeth; left + right)	4 (7) + 8	(4–5) + (8–9)	? + 8	
MxV (number of teeth; left + right)	1 + 1	1 + 1	? + 1	
Branchiae – first chaetiger emerges[a]	35 (4 – 63)	(30 – 49)	30	(14 – 46)
Branchiae – last chaetiger emerges	About 20 chaetigers before pygidium	About 20 – 25 chaetigers before pygidium	Not mentioned	About 20 – 25 chaetigers before pygidium
Branchiae – max. filaments[a]	11 (14)	6	6	6
Post-chaetal lobe – shape anteriorly	First chaetiger: sub-conical but gradually becoming subtriangular, low and broad and slightly bilobed after chaetiger 100	Pre- and post-chaetal lobes continuous around dorsal edge of neuropodium	Low and broad	Low and broad
Pectinate chaetae – first present[a]	Present from chaetiger 5 (3)	Present on mid-body chaetigers (~100)	Present beginning from the mid-section towards posterior	Present on first few chaetigers
Pectinate chaetae – symmetry[a]	Four types – 1. Symmetrical, narrow shaft with thin teeth (~30) 2. Asymmetrical with thinner teeth (>30) and broad shaft 3. Asymmetrical with thinner teeth (<30) with narrow shaft 4. Wide body with wide teeth (~8)	Three types of asymmetrical (no. teeth): 1. Coarse teeth (~30) with broad shaft 2.Thinner teeth (~30) with narrow shaft 3. Wide body with wide teeth (~8)	Pectinate (symmetrical?) on anterior segment and fan chaetae (asymmetrical?) on posterior segments	Asymmetrical pectinate chaetae throughout
Pectinate chaetae: no. of teeth (anterior)	21	Not mentioned	Not mentioned	(15 – 25)
Pectinate chaetae – no. of teeth (midbody and posterior chaetigers)	44	Up to 50 teeth	Fan chaetae – ~35 teeth	(30 – 40)
Pectinate chaetae – no. per parapodia[a]	(1 – 6)	(1 – 10)	No info on pectinate chaetae, but fan chaetae are ≥ 2	(0 – 2)

Characteristics	***Marphysa moribidii* sp. nov. (present study)**	***Eunice (Marphysa) mossambica* (Lectotype; Fauchald 1987 and present study)**	***Nauphanta novaehollandiae (Marphysa cf. mossambica* (*sensu* Fauchald 1987))**	***Marphysa* cf. *mossambica* (*sensu* Glasby and Hutchings, 2010)**
Pectinate chaetae – outer teeth	Slightly longer than inner teeth	Long thickened superior edge	Pectinate chaetae – similar length for outer and inner teeth	Slightly longer than inner teeth, one longer than other
Sub-acicular limbate capillaries	Present	Present	Present	Present
Sub-acicular limbate capillaries – first present	Chaetiger 1	Chaetiger 1	Chaetiger 1	Chaetiger 1
Sub-acicular hooks	Absent (present)	Present	Present	Present
Sub-acicular hooks – tips	Hooded, bidentate	Bidentate	Bidentate	Bidentate
Sub-acicular hooks – first chaetiger	(44 – 100); irregular pattern	(58 – 73); irregular pattern	44; irregular pattern	58 (23 – 68)
Sub-acicular hooks – max no.	2	Not mentioned	Not mentioned	1
Aciculae – max no.	4	Not mentioned	2	4
Aciculae – colour	Dark brown	Dark to light brown	Brown	Brown
Pygidium	2 pairs of cirri located on the ventral side (one long pair, one short-and-small pair)	Not mentioned	Not mentioned	Not mentioned

Biology and ecology. Marphysa moribidii sp. nov. is dioecious and iteroparous. This can be seen by the presence of oocytes of varying sizes in every month (Idris et al., in prep.). Sexual dimorphism is not present in *M. moribidii* sp. nov. The population at the type locality (Morib mangrove) (fig. 1) is unevenly distributed and can be found down to depths of about 450 mm from the surface in the mangrove area with *Rhizophora apiculata*, *Avicennia alba* and *Sonneratia caseolaris*. The new species is a sub-surface deposit feeder based on analysis of its intestinal contents (Idris et al., in prep.).

Distribution. Along the Straits of Malacca, Singapore, in the mangrove area with *Rhizophora* spp., *Avicennia alba* and *Sonneratia caseolaris*.

Economic exploitation. M. moribidii sp. nov. is one of the polychaete species harvested as bait worms in Peninsular Malaysia (Idris and Arshad, 2013). The species is harvested and sold in Malaysian states along the Straits of Malacca, except Perlis. Five to ten individuals of *M. moribidii* sp. nov. (mostly incomplete) are sold for MYR10 (~US$3). Although the *M. moribidii* sp. nov. fishery is currently unregulated and undocumented, selling of this species is believed to have been conducted for many years. Most bait diggers harvest the species either on a part-time basis (mainly on weekends due to low demand on weekdays, except for school and public holidays) or for personal use. Some bait diggers also sell worms to fishing shops or are contracted by them to collect the worms. Fortunately, harvesting and selling of *M. moribidii* sp. nov. is very localized since the worms do not live outside their natural habitat for a long period (2 – 3 days), and coelomic fluid from broken specimens has been found to accelerate the mortality of other worms when packed together (pers. obs.).

Marphysa mossambica (Peters, 1854)

Marphysa mossambica Gravier, 1900: 267, pl. 14, figs 89–90, text figs 137–139.—Crossland, 1903: 139–140, pl. 15, figs 7–10.—Day 1967, 395, fig. 17.5 i–m.

Synonymy.

Eunice mossambica Peters, 1854: 612.

Nauphanta novaehollandiae Kinberg, 1865: 564; 1910: 43, pl. 16, fig. 23, 23B, C, F ,G.

Marphysa simplex Treadwell, 1922: 151–152, text-fig. 39, pl. 5, figs 8–12.

Nauphanta mossambica Fauchald, 1987: 376–378, fig. 1.

Figure 6.

Material examined. Lectotype. ZMB 4005 – complete, female. Paralectotypes. (6): ZMB 47 and ZMB F2046, all specimens were collected at Moçambique, coll. Peters 1854.

Remarks. We re-examined the lectotype (ZMB 4005) and the associated SEM stubs used in Zanol et al. (2014). The anterior section was photographed while the following parapodia have been mounted: 2, 32, 96, 160, 224 and 252. The anterior section of *M. mossambica* is light brown and the white spots absent (fig. 6A). The limbate chaetae are observed throughout the chaetigers (fig. 6B). We observed that *M. mossambica* has three types of pectinate (described as 'fan' by Fauchald, 1987) chaetae: two asymmetrical and one with few teeth (figs. 6C, D, E), confirming the observations of Zanol et al. (2014). The first asymmetrical type consists of chaetae with about 30 teeth with broad shaft (figs. 6C, D), while the second asymmetrical type also has about 30 teeth but with shaft narrower than the first type (fig. 6E). The other type, with only eight to nine large saw-like teeth (identified as 'wide-toothed pectinate' by Zanol et al. 2014) is situated basally to the asymmetrical pectinate chaetae (figs. 6C, D). This type of pectinate chaeta only appears in posterior chaetigers (found in chaetigers 224 and 252) at the base of the chaetal fascicle and is easily obscured by limbate chaetae and other pectinate chaetae. We were able to observe this type of chaeta under SEM (also observed under SEM by Zanol et al. 2014) and only under light microscope with careful adjustment, which may explain why Fauchald (1987) failed to describe them when he re-examined the lectotype. Fauchald (1987, his figs. 1b, c) illustrates two types of pectinate chaetae, varying in the number of teeth—one with about 20 and one with 40, neither markedly asymmetrical, although, as seen in figs. 6C – E, they are clearly asymmetrical. These two types of pectinate chaetae are present from the early mid-body (>30 segments), which contradicts an earlier observation by Fauchald (1987), that they do not occur until after parapodia 100.

Glasby and Hutchings (2010) recorded *M. mossambica* from various locations in Australia, but did not examine the lectotype, relying on Fauchald's (1987) revised description. Re-examination of other Australian material from Queensland identified as this species (AM W33021) under the SEM did not reveal the pectinate chaetae with only 8 – 9 teeth, and we now believe that the Australian material listed by Glasby and Hutchings (2010) needs to be re-examined as it may represent another undescribed species in this complex (we are now referring to it as *Marphysa* cf. *mossambica* until further studies are completed).

Discussion

With the exception of the pectinate chaetae types and characters on *M. mossambica*, all three species (*M. moribidii, M. mossambica* and *M.* cf. *mossambica*) are difficult to differentiate due to the subtlety of their differences. Details of *M. moribidii* and comparisons with the other two sibling species are shown in table 1.

Our study highlights the need for obtaining complete specimens to allow examination of parapodia from all sections of the animal. This is probably why specimens from the western part of the Indian Ocean are still being identified as *M. mossambica*. The specimens nearest to *M. mossambica* were identified in Singapore (Chan, 2009), India (Nicobar Is.) (Rajasekaran and Fernando, 2012) and the Taiwan Straits (Paxton and Chou, 2000). We suggest that specimens from these locations as well as other parts of the Western Pacific should be re-examined. In particular, the posterior chaetigers need to be studied in order to determine the presence or absence of wide pectinate chaetae with wide teeth.

Although *Marphysa* is a species-rich genus (Orensanz, 1990), some species have been described as having a cosmopolitan distribution. One is *M. sanguinea* Montagu, 1813, which has been reported from all oceans of both northern and southern hemispheres, except for the polar regions (see Day, 1967; Miura, 1977; Gathof, 1984; Paxton and Chou, 2000; Prevedelli et al., 2007). However, Hutchings and Karageorgopoulos (2003), as well as Lewis and Karageorgopoulos (2008), have challenged the cosmopolitan status of *M. sanguinea.* Hutchings and Karageorgopoulos (2003) suggest that the distribution of *M. sanguinea* is restricted to northern Europe, and that records from other parts of the world should be checked. Certainly, the records of *M. sanguinea* from South Africa have been found to represent another species (Lewis and Karageorgopoulos, 2008), and this has been confirmed both morphologically and molecularly.

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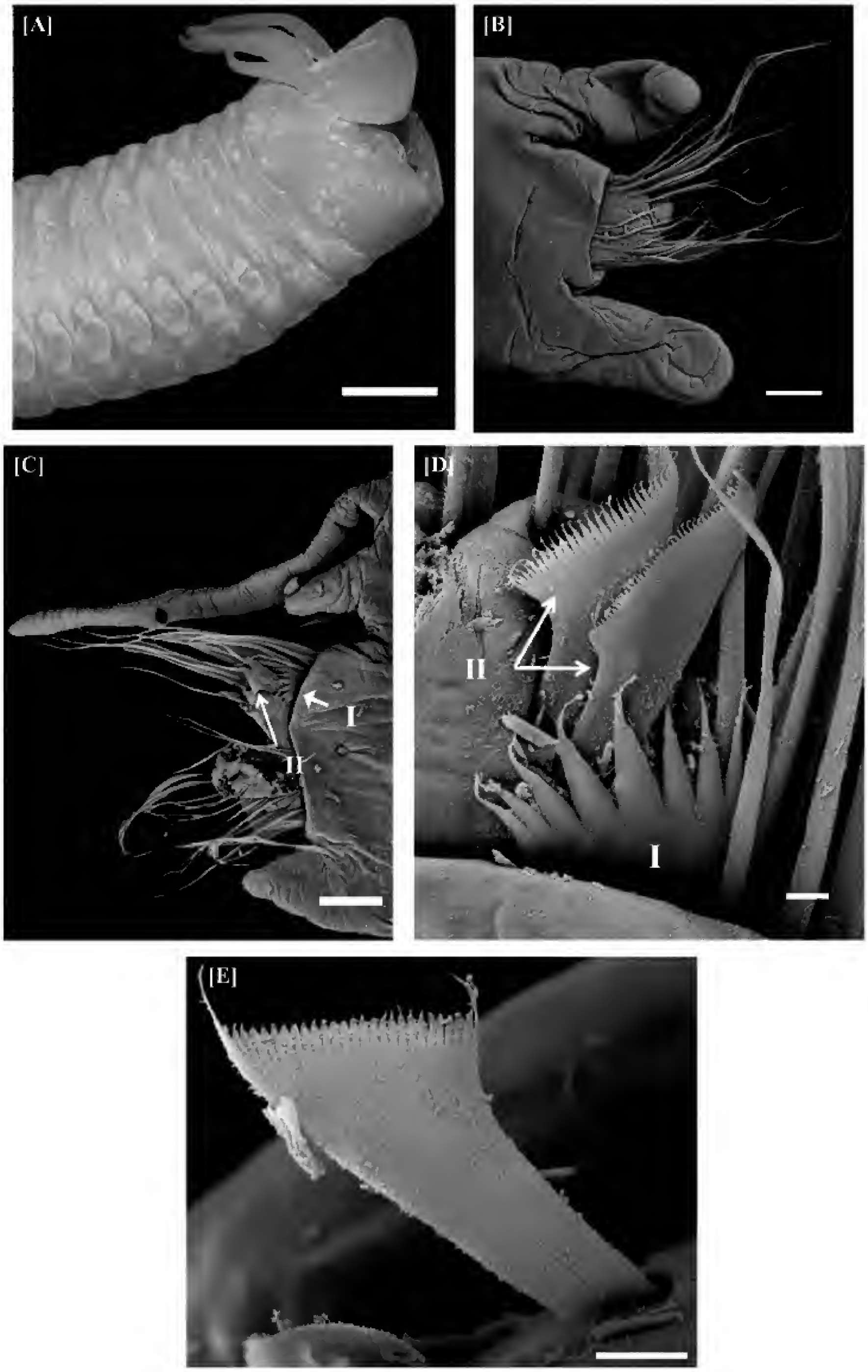

Figure 6. *Marphysa mossambica*. [A] anterior section, lateral view; [B] limbate chaetae, unique character for Group. A, chaetiger 2; [C] two types of pectinate chaetae (arrows), I: wide teeth and wide body, and II: asymmetrical pectinate with broad shaft, chaetiger 224; [D] details of pectinate chaetae, I: wide teeth and wide body, and II: asymmetrical pectinate with broad shaft, showing the cryptic position of wide teeth and wide body chaeta, chaetiger 224; [E] another type of pectinate chaeta, asymmetrical pectinate with narrow shaft. chaetiger 224. Scale bars: [A] = 5 mm; [B, C] = 100 μm; [D, E] = 10 μm. All images are from the lectotype (ZMB 4005).

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Written in stone: history of serpulid polychaetes through time

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Abstract

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Although the fossil record of annelids in general is poor, calcareous tube-building Serpulidae are a notable exception. The "stumbling block" of understanding the serpulid fossil record is obtaining reliable taxonomic interpretations of fossil tubes based on morphology. Luckily, serpulid tubes demonstrate high variety of ultrastructures and nonuniform mineralogical composition, which can be used as new tools for decrypting the fossil record. Ancient Late Ediacaran (580-541 Ma) and Paleozoic (541-252 Ma) rocks contain diverse tubicolous fossils that have often been erroneously interpreted as annelids, and serpulids, in particular. Palaeozoic to Middle Jurassic coiled spirorbiform tubes, often referred to as *Spirorbis*, had been shown to be microconchids, a group of probable lophophorate affinity. The most ancient records of unequivocal serpulids date back to the Middle Triassic (~244 Ma) of the Mesozoic, and from the Earliest Jurassic (~200 Ma) fossil serpulids become common. From the latest Jurassic (~146 Ma) serpulids colonised hydrocarbon seep environments and possibly also penetrated the deep sea. Concerted efforts of paleontologists and zoologists are needed for further understanding of serpulid evolutionary history. The serpulid fossil record can become a valuable instrument for calibration of "molecular clocks" in polychaetes, which would allow dating not only divergence events in serpulids, but also in annelid groups that lack a representative fossil record.

Keywords Annelida, Polychaeta, Serpulidae, biomineralisation, fossil record, tube ultrastructure, mineralogy

Introduction

Polychaetes are mostly soft-bodied animals with a very poor paleontological record. Imprints of soft-bodied animals are rare and only known from a limited number of localities with exceptional preservation (so called "Lagerstätten"). The most important among them are the Cambrian Burgess Shale (505 Ma; Conway Morris, 1979; Eibye-Jacobsen, 2004), the Devonian Hunsrück Slate (405 Ma; Briggs and Bartels, 2010), the Carboniferous Mazon Creek fauna (310 Ma; Fitzhugh et al., 1997), and the Cretaceous Hakel polychaete fauna (~95 Ma; Bracchi and Alessandrello, 2005). The oldest known annelid fossils are polychaetes from the Cambrian (Vinther et al., 2011) and the oldest known fossil polychaete is *Phragmochaeta canicularis* Conway Morris et Peel, 2008 from the Early Cambrian Sirius Passet (518 Ma) fauna.

In the paleontological record, polychaete fossils are dominated by biomineralised tubes and, sometimes, fossilised jaws, known as scolecodonts (e.g. Hints and Eriksson, 2007). Although many polychaetes build muddy or mucous (Sabellidae), chitinous (e.g. Chaetopteridae, Siboglinidae), agglutinated (e.g. Pectinariidae, Sabellariidae) or calcareous tubes, only tubes made of calcium carbonate have good chances to be preserved. Of the three polychaete families known to build calcareous tubes (Serpulidae, Sabellidae, and Cirratulidae), serpulids are obligatory calcareous tube builders, whereas in cirratulids and sabellids calcareous tubes are restricted to a single genus in each family (Perkins, 1991; ten Hove and van den Hurk, 1993; Fischer et al., 1989; 2000; Vinn et al., 2008a; Vinn, 2009). Not surprisingly, serpulids have the best fossil record among all annelids, being represented mainly by tubes, and, to a lesser degree, by calcified opercula.

Serpulids are common on hard substrata in all marine habitats at all depths, being an important element of the encrusting biota in Recent seas. They are important fouling organisms and can also form reefs. Fossil serpulid tubes were first described over 300 years ago, in "Oryctografia Norica" by

the German doctor Johann Jakob Baier (1708) as "Tubus vermicularis fossilis". Despite this, geologists and paleontologists traditionally pay little attention to the group, partly because of the perceived opinion of its small potential value in stratigraphy and reconstructing paleoenvironments. There are several large reviews of serpulid faunas of different geological periods (e.g. Rovereto, 1899; 1904; Brünnich Nielsen, 1931; Parsch, 1956; Schmidt, 1955; Lommerzheim, 1979; Jäger, 1983; 1993; 2005), but only few papers (e.g. Jäger 1983, 1993, 2005) discuss evolution and geological history of fossil serpulids. The only comprehensive overview of the entire serpulid fossil record in the Phanerozoic by Götz (1931), and a short summary by Regenhardt (1964) are now clearly outdated, and the most recent review (Vinn and Mutvei, 2009) focuses mainly on false serpulids from the Paleozoic.

The aims of the present paper are: 1) to outline the serpulid fossil record, including discussion of some serpulid-like tubicolous fossils; 2) to discuss the current state of knowledge of serpulid paleontology and 3) to indicate directions of future research in the evolutionary history of serpulids.

1. Current state of serpulid systematics and phylogeny

According to the most recent review of serpulid taxonomy (ten Hove and Kupriyanova, 2009), the family comprises 46 genera with about 350 extant species. This, however, does not include about 140 species from the nominal subfamily Spirorbinae, arranged in 24 genera (Ippolitov and Rhzavsky, 2014). Serpulidae Rafinesque, 1815 was not subdivided into subfamilies until Chamberlin (1919) established the subfamily Spirorbinae for small-sized serpulids having tubes coiled into flat spirals. Later Rioja (1923) placed hypothetically primitive species with a pinnulated operculum-bearing radiole or without operculum into the subfamily Filograninae. Pillai (1970) elevated Spirorbinae to the family Spirorbidae, which was widely accepted until phylogenetic data, both based on morphology and molecular analyses (e.g. Kupriyanova, 2003; Kupriyanova et al., 2006; Lehrke et al., 2007) indicated that spirorbins are nested inside Serpulidae. Thus, the family status of Spirorbinae is not justified because recognition of Spirorbidae would make Serpulidae *sensu stricto* a paraphyletic group. All phylogenetic molecular analyses indicate that neither traditional Serpulinae, nor Filograninae are monophyletic and that spirorbins are close to "filogranin" taxa (Kupriyanova et al., 2006; 2009; Lehrke et al., 2007; Kupriyanova and Nishi, 2010), with the result that the traditional subfamilies were abandoned. The analyses inferred two major clades (tentatively termed A and B) within Serpulidae (fig. 1). Clade A comprises two subclades: clade AI, the "*Serpula*-group" (with the genera *Serpula, Crucigera, Hydroides*), and clade AII, the "*Spirobranchus*-group" (with, amongst others, the genera *Spirobranchus*, *Ficopomatus* and *Ditrupa*). Clade B included clade BII (the monophyletic subfamily Spirorbinae) as sister group to clade BI, the "*Protula*-group" (with amongst others the genera *Protis, Protula* and *Vermiliopsis*). Relationships within clade AI were further briefly studied by Kupriyanova et al. (2008). No molecular spirorbin phylogeny is currently available, but Macdonald (2003) proposed a hypothesis based on morphological data.

2. Decrypting the serpulid fossil record: where we are

2.1. The stumbling block in fossil record interpretation

The main problem of serpulid paleontological record is obtaining reliable taxonomic interpretations of fossil tubes. Starting with Rovereto (1899; 1904) for the Cenozoic and Regenhardt (1961) and all subsequent authors for the Mesozoic, attempts were made to determine fossil tubes according to the classification used for Recent species (e.g. Lommerzheim, 1979; 1981; Jäger, 1983; 1993; 2005; Radwańska, 1994a; 2004; Ippolitov, 2007a; 2007b; Jäger and Schubert, 2008; Schlögl et al., 2008; Vinn and Wilson, 2010). However, classification of extant serpulids is based on body and chaetal characters, while little attention is paid to the tube morphology. While a tube is important for protection, it is not integrated with the animal body, and thus, does not constitute a genuine exoskeleton (Regenhardt, 1964; Weedon, 1994; Seilacher et al., 2008). Adaptive evolution of tubes is relatively independent of that of the soft tissue, resulting in relatively weak correlations between tube and body characters used by zoologists for classification of Recent forms. This probably explains why polychaete tubes, unlike mollusc shells, have not become very important for taxonomy. Some Recent genera have very distinct tubes (e.g. *Janita*, *Vitreotubus*, *Neomicrorbis*, *Placostegus*, *Ditrupa*) easily recognizable in fossil state (see section 2.2). In others (e.g. *Bathyvermilia*, part of *Filogranula*, *Semivermilia*, *Pseudovermilia*, *Pyrgopolon*, *Spiraserpula*), tube morphology is important for species distinction, but reliable generic attribution based on tubes alone is difficult due to high intra-generic variability. Moreover, tubes of some speciose genera often show little or no interspecific variability (*Spirobranchus*, *Serpula*, and *Hydroides*) or have a very simple tube morphology (e.g. *Apomatus*/*Protula*, *Hyalopomatus*), making their recognition in the fossil state problematic. Most species of the largest genus *Hydroides* comprising around 100 extant species have uniform tubes with a flattened upper surface, sometimes with two or three indistinct keels.

Such genera that are "problematic" from the paleontological point of view comprise about 55% of the Recent non-spirorbin serpulids (Table 1). In Spirorbinae the situation is even worse, as normally no Recent genera, except for a very distinct questionable spirorbin *Neomicrorbis* and the peculiar fossil genus *Bipygmaeus*, can be confidently determined by tube morphology alone. Reasonably confident determinations of fossil spirorbins are based mainly on opercula associated with tubes (Lommerzheim, 1981; Jäger, 1993; 2005). However, because preservation of opercula is uncommon, determinations by tubes inevitably remains the main means of study of fossil spirorbins.

Paleontologists are restricted in their interpretations to "easily recognisable" genera. Other fossil species are tentatively classified within known Recent genera, assigned to exclusively "fossil" genera, or conventionally treated as "*Serpula*?" (Lommerzheim, 1979; Jäger, 1993; 2005). As a result, zoologists are skeptical about most generic affinities proposed by paleontologists based on tubes. This leads to a paradoxical situation when despite diverse and abundant fossils, zoologists lack reliable paleontological data for understanding the

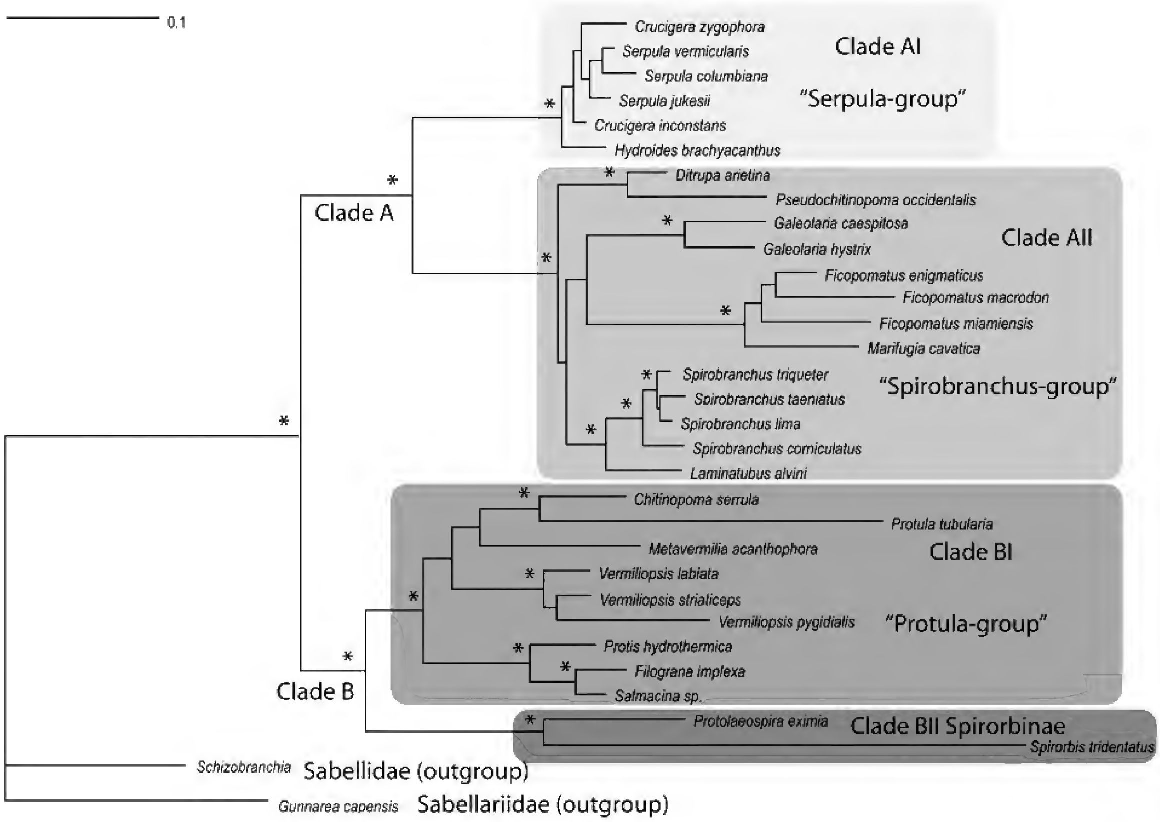


Figure 1. A hypothesis of phylogenetic relationships within Serpulidae (a Bayesian majority rule consensus phylogram of the combined 18S and 28S rDNA serpulid sequence data; modified from Kupriyanova et al., 2009). Nodes with posterior probabilities of 1.0 are indicated by "*".

evolutionary history of the group, while paleontologists are restricted in their geological, paleoecological, and biogeographical interpretations because no direct comparison of fossils with Recent taxa is possible. Currently described fossil serpulids are grouped in about 50 genera, 40% of which are taken from Recent zoology (Table 1), and ~60% are used exclusively for fossil material (Table 2). Whether these fossil genera are truly extinct taxa, or should be synonymised with extant genera (and *vice versa*), is not always obvious. The current interrelation of Recent and fossil genera (Table 1) shows that although many extant serpulid genera are recognised in fossil state, the attribution of fossil tubes is often problematic.

2.2. Tube morphology: how helpful is it for understanding fossil record?

Comparative morphology of fossil tubes remains the major tool of serpulid paleontology. The main characters allowing recognition of Recent genera in the fossil state (fig. 2) are type of aggregation, type of coiling/curving, attachment to the substrate, external sculpture, expansion rate, presence of internal tube structures (ITS), development of attachment structures, wall opacity/transparency, appearance, size, and opercular morphology.

Aggregations. Dense aggregations of serpulid tubes can be formed either by asexual reproduction or as a result of gregarious larval settlement. Asexual budding results in branching "pseudocolonies" *sensu* Nishi and Nishihira (1994) of *Filograna/Salmacina* (fig. 2D) that are easily recognizable as fossils. Gregarious larval settlement leading to dense aggregations is typical for Recent *Ficopomatus* (fig. 2L), *Serpula,* and *Hydroides*. This process is also a key to reef formation by serpulids. In the case of *Filogranella* it is not clear which factors are the main contributors to its reef-forming (Hoeksema and ten Hove, 2011), although aggregations may reach huge sizes. For some fossil taxa that sometimes build aggregations, such as *Parsimonia*, a close relationship to *Serpula* was proposed (Regenhardt, 1961).

Free and attached tubes. Normally tubes are attached to the hard substrate at least partially, but some serpulids e.g. *Ditrupa, Bathyditrupa, Nogrobs* (fig. 2A, B, J, respectively) and, occasionally, species in other genera (e.g. *Serpula crenata*

Table 1. Recent serpulid genera and their fossil record. The list of Recent non-spirorbin genera follows ten Hove and Kupriyanova (2009) data with modifications, the list of Recent spirorbin genera and species number is after Ippolitov and Rzhavsky (2014: Tab. 1). Dating of the most ancient finds does not reflect origin time as due to the scarcity of fossil record most taxa are probably older than indicated. The number of fossil species for each genus is approximate, as most of fossil species described as "*Serpula*" in older publications need to be revised. Absolute ages here and in the text are provided according to the official site of the International Commission of Stratigraphy www.stratigraphy.org/GSSP/index.html, accessed 10-12-2013. Designations: *genera with fossil type species; **some extant species recognised also as fossils in sub-Recent (Pliocene-Holocene) sediments; †taxa originally used in paleontological literature only (extinct genera).

Genus (including most common synonyms and subgenera)	Number of extant species	Number of fossil species	Most ancient fossil finds and their age	Tube characters allowing recognition in fossil state
SABELLIDAE				
*Glomerula** Brünnich Nielsen, 1931 =*Calcisabella* Perkins, 1991, =†*Cycloserpula* Parsch, 1956, =†*Omasaria* Regenhardt, 1961	1	7+	Late Carboniferous (323-304 Ma; present paper) or Late Hettangian (200 Ma; Jäger, 2005)	glomerate coiling; very slow expansion; absence of basal cementing flanges
NON-SPIRORBIN SERPULIDAE				
Apomatus Philippi, 1844	7	-	-	not recognised
Bathyditrupa Kupriyanova, 1993a	1	?	?Late Pliensbachian (~185 Ma; Behrendsen, 1891);?Late Albian (~105 Ma; Jäger, 2005)	unattached tusk-shaped tubes with quadrangular cross-section. Maybe synonym of †*Nogrobs (Tetraditrupa)* (see Jäger, 2005) or †*Nogrobs (Tetraserpula)* (see Ippolitov, 2007a).
Bathyvermilia Zibrowius, 1973	5	1?	??Late Sinemurian ("*Serpula*" *etalensis* (Piette, 1856); ~194 Ma)	long free anterior part with characteristic frequent peristomes
Chitinopoma Levinsen, 1884	3-4	-	-	not recognised
Chitinopomoides Benham, 1927	1	-	-	not recognised
Crucigera Benedict, 1887	5	-	-	not recognised
Dasynema de Saint-Joseph, 1894	1	-	-	not recognised
Ditrupa Berkeley, 1835 =†*Acerrotrupa* Yu et Wang, 1981, =†*Sinoditrupa* Yu et Wang, 1981	2	1+	Danian (65 Ma; Jäger, 1993)	unattached tusk-shaped tubes with circular cross-section
Ficopomatus Southern, 1921	5	-	-	not recognised.
Filograna Berkeley, 1835	1	5+	Late Anisian (244 Ma; Senowbary-Daryan et al., 2007)	pseudocolonial; small-sized; individual tubes packed in branching bundles. Indistinguishable from *Salmacina*
Filogranella Ben-Eliahu et Dafni, 1979	1(3?)	-	-	not recognised
Filogranula Langerhans, 1884 ?=†*Flucticularia* Regenhardt, 1961	6	6**	late Early Toarcian (~180 Ma; Jäger, unpubl.; Ippolitov, 2007a)	sculpture; size; aperture with spines
Floriprotis Uchida, 1978	1	-	-	not recognised
Galeolaria de Lamarck, 1818	2	1	Cenomanian (100 Ma; Lommerzheim, 1979)	sculpture (massive median bicarinate keel), cross-section
Hyalopomatus Marenzeller, 1878	11-12	-**	-	not recognized
Hydroides Gunnerus, 1768	89	?**	?Middle Paleocene (~60 Ma; Lommerzheim, 1981); or Middle Miocene (~15 Ma; Schmidt, 1955)	flattened upper side, usually bordered by keels, coiling tendency
Janita de Saint-Joseph, 1894	1	-**	?Cenomanian (100 Ma; Lommerzheim, 1979); or ?Badenian (15 Ma; Radwańska, 1994a)	not recognised confidently

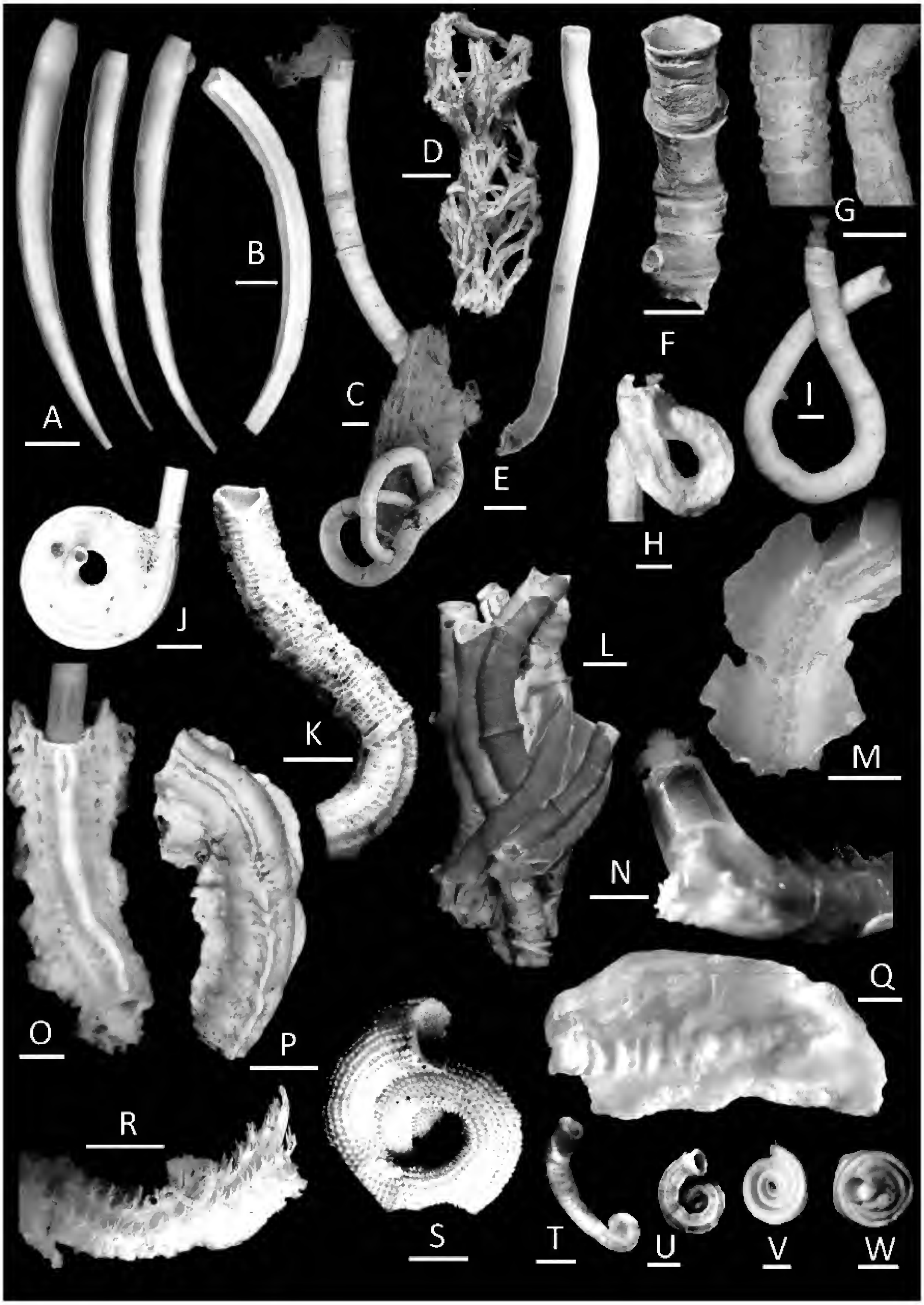
Genus (including most common synonyms and subgenera)	Number of extant species	Number of fossil species	Most ancient fossil finds and their age	Tube characters allowing recognition in fossil state
Josephella Caullery et Mesnil, 1896	1	2	?earliest Cenomanian (100 Ma; Lommerzheim, 1979)	size, very slow expansion
Laminatubus ten Hove et Zibrowius, 1986	1	-	-	not recognized
Marifugia Absolon et Hrabě, 1930	1	-**	Pliocene/earliest Pleistocene (2.5-3.6 Ma; Bosák et al., 2004)	the only extant species found in fossil state
Metavermilia Bush, 1905 subgen.: †*Vepreculina* Regenhardt, 1961	14	7+	Late Rhaetian (205 Ma; Jäger, 2005); or Late Callovian (165 Ma; Ippolitov, 2007a)	sculpture, size, growth rate
Microprotula Uchida, 1978	1	-	-	not recognized
Neovermilia Day, 1961 =†*Proliserpula* Regenhardt, 1961	6	3+**	Late Oxfordian (158 Ma; Radwańska, 2004)	size, sculpture, attachment structures morphology
*Nogrobs** de Montfort, 1808 =*Spirodiscus* Fauvel, 1909, =†*Ditrupula* Brünnich Nielsen, 1931, ?=†*Glandifera* Regenhardt, 1961, ?=†*Tubulostium* Stoliczka, 1868; subgen.: (?)†*Tetraditrupa* Regenhardt, 1961; (?)†*Tetraserpula* Parsch, 1956 [Interrelations between all subgenera remain uncertain]	1	10+	Late Pliensbachian (~185 Ma; see Jäger, 2005) – non-spiral forms of subgenus *Tetraserpula*; Late Toarcian (~176 Ma; Jäger, 2005) – spiral forms of *Nogrobs s. str.*	spiral coiling, quadrangular cross-section
Omphalopomopsis de Saint-Joseph, 1894	1	-	-	not recognised
Paraprotis Uchida, 1978	1(2?)	-	-	not recognised
Paumotella Chamberlin, 1919	1	-	-	not recognised
Placostegus Philippi, 1844 =†*Eoplacostegus* Regenhardt, 1961	7	7+	Late Oxfordian (158 Ma; Radwańska, 2004)	cross-section, aperture with spines, size, growth mode
Pomatostegus Schmarda, 1861	3	-	-	not recognised
Protis Ehlers, 1887	6-7	-	-	not recognised
Protula Risso, 1826 =*Membranopsis* Bush, 1910; subgen.: †*Longitubus* Howell, 1943	?24	3+**	Early Albian (~113 Ma; see Jäger, 2005)	medium to large-sized tubes, often growing upwards from the substrate; no sculpture
Pseudochitinopoma Zibrowius, 1969	2	2	Early Oxfordian (163 Ma; Ippolitov, unpubl.)	size, well-developed transverse sculpture
Pseudovermilia Bush, 1907	10	2?	?Cenomanian (100 Ma; Lommerzheim, 1979); or ?Burdigalian (20 Ma; Jäger and Schneider, 2009)	size, sculpture
*Pyrgopolon** de Montfort, 1808 =*Sclerostyla* Mørch, 1863, =†*Falcula* Conrad, 1870, =†*Hexaserpula* Parsch, 1956, =†*Hepteris* Regenhardt, 1961; subgen.: †*Hamulus* Morton, 1834; †*Turbinia* Michelin, 1845 (=†*Pyrgopolopsis* Rovereto, 1904); †*Ornatoporta* Gardner, 1916; †*Septenaria* Regenhardt, 1961	3	15+	Barremian (128 Ma; Jäger, 2011)	tube size, expansion rate; growth mode; sculpture

Genus (including most common synonyms and subgenera)	**Number of extant species**	**Number of fossil species**	**Most ancient fossil finds and their age**	**Tube characters allowing recognition in fossil state**
Rhodopsis Bush, 1905	2	-	-	not recognised
Salmacina Claparède, 1870	11	?	?	indistinguishable from *Filograna*
Semivermilia ten Hove, 1975	8	?1	?Badenian (15 Ma; Radwańska, 1994a)	not recognised confidently
Serpula Linnaeus, 1758 (?) subgen.: †*Cementula* Brünnich Nielsen, 1931	29	?**	?Cenomanian (100 Ma; Jäger, 2005); Paleogene (~66 Ma) to Recent	most fossil species are described under this generic name. True *Serpula* ("s. str.") determined by two/three keeled tubes. Morphological specification is too poor to allow confident recognition, so precise number of fossil species is not clear now.
*Spiraserpula** Regenhardt, 1961	18	6+	Late Callovian (164 Ma; Ippolitov, 2007b)	coiling type, ITS
Spirobranchus de Blainville, 1818 =*Pomatoceros* Philippi, 1844, =*Pomatoleois* Pixell, 1913	26+	2+**	?Cenomanian (100 Ma; Lommerzheim, 1981, by opercula); Middle Paleocene (~60 Ma; Lommerzheim, 1981)	large size; subtriangular section, opercular morphology
Tanturia Ben-Eliahu, 1976	1	-	-	not recognised
Vermiliopsis de Saint-Joseph, 1894 =†*Peraserpula* Regenhardt, 1961	13-19	4+	Late Callovian (164 Ma; Vinn and Wilson, 2010)	trumpet-shaped peristomes, sculpture, fast growth
Vitreotubus Zibrowius, 1979	1	-**	-	not recognised
SPIRORBINAE				
Amplicaria Knight-Jones, 1984	1	-	-	not recognised
Anomalorbis Vine, 1972	1	-	-	not recognised
Bushiella Knight-Jones, 1973	13(14?)	-	-	not recognised
Circeis de Saint-Joseph, 1894	6	3	Middle Paleocene (~60 Ma; Lommerzheim, 1981)	some species described by opercula with good confidence; tubes – by coiling direction; sculpture; with poor confidence
Crozetospira Rzhavsky, 1997	1	-	-	not recognised
Eulaeospira Pillai, 1970	2	1	??Cenomanian (100 Ma; Lommerzheim, 1979)	low confidence
Helicosiphon Gravier, 1907	1	-	-	not recognised
Janua de Saint-Joseph, 1894	1	3**	??Cenomanian (100 Ma; Lommerzheim, 1979); Middle Paleocene (~60 Ma; Lommerzheim, 1981)	some species described after opercula; some based on tubes, with low confidence
Knightjonesia Pillai, 2009	1	-	-	not recognised
Leodora de Saint-Joseph, 1894	1	-	-	not recognised
Metalaeospira Pillai, 1970	4	2	??Cenomanian (100 Ma; Lommerzheim, 1979) or Middle Paleocene (~60 Ma; Lommerzheim, 1981)	low confidence for ancient Paleocene species determined by opercula

Genus (including most common synonyms and subgenera)	Number of extant species	Number of fossil species	Most ancient fossil finds and their age	Tube characters allowing recognition in fossil state
Neodexiospira Pillai, 1970	10(11?)	5+	?Late Barremian (~126 Ma; Jäger, 2011), Maastrichtian (72 Ma)	operculum; tube sculpture, coiling direction; relatively good confidence for most ancient species
Nidificaria Knight-Jones, 1984	8	-	-	not recognised
Paradexiospira Caullery et Mesnil, 1897	3(4?)	-	-	not recognised
Paralaeospira Caullery et Mesnil, 1897	10	1	Middle Paleocene (~60 Ma; Lommerzheim, 1981)	operculum morphology, coiling direction, sculpture
Pillaiospira Knight-Jones, 1973	3	-	-	not recognised
Pileolaria Claparède, 1868	21(22?)	?**	?Late Barremian (~126 Ma; Jäger, 2011)	low confidence
Protolaeospira Pixell, 1912	12	-**	-	not recognised
Protoleodora Pillai, 1970	4	-	-	not recognised
Romanchella Caullery et Mesnil, 1897	8	-	-	not recognised
Simplaria Knight-Jones, 1984	3	-	-	not recognised
Spirorbis Daudin, 1800	15	?**	??Cenomanian (100 Ma; Lommerzheim, 1979)	most of fossil material described in older publications is conventionally placed under this name
Vinearia Knight-Jones, 1984	3	-	-	not recognised
GENERA OF UNCERTAIN NATURE (DOUBTFUL SPIRORBINAE)				
*Neomicrorbis** Rovereto, 1903 =†*Granorbis* Regenhardt, 1961; =†*Spirorbula* Brünnich Nielsen, 1931	1	7+	?Late Bathonian (~167 Ma; Jäger, 2005); Late Berriasian (~142 Ma; Ippolitov, unpubl.)	coiling to both directions, sculpture, large size

(Ehlers, 1908), *S. israelitica* Amoureux, 1976, and *Pyrgopolon differens* (Augener, 1922)), are secondary free-living on soft substrate as adults, while larvae attach to smallest objects. Among fossils similar free-lying tubes are known in such genera as *Tetraserpula*, *Tetraditrupa*, *Triditrupa*, *Pentaditrupa*, and *Nogrobs*, as well as in large number of highly diversified spirally coiled forms (*Rotularia*-shaped genera, *Conorca*, *Orthoconorca*).

Tube shape and coiling. General tube shape in most genera is undetermined, resulting in a variety of straight, irregularly twisted or coiled tubes within a genus or even species. Some, however, have a determined tube shape, e.g. tusk-shaped *Ditrupa*, *Bathyditrupa* and all spirally coiled taxa (fig. 2A, B, J, S-W). *Spiraserpula*, known both as Recent and fossil, tends to alternate spirally coiled and irregularly curved tube segments. Coiling mode (spirals attached to substrate or growing over each other) and direction (clockwise only, anticlockwise only, or both) are the most important characters for both extant and extinct forms. Obligatory trochospiral coiling, where coiling direction can be both clockwise and anti-clockwise within a species, is characteristic only of some fossil genera such as *Conorca*, *Protectoconorca*, *Orthoconorca*, and *Rotularia*-shaped genera (Regenhardt, 1961; Jäger, 1983; 1993). The proportion of tubes coiled in each direction can be either constant within a species or vary intraspecifically for material of slightly different geological ages (Jäger, 1983: Tab. 3-5). There is also a tendency to have one coiling direction strongly dominant (e.g. in some *Orthoconorca*, *Protectoconorca* and *Rotularia*). Spirorbins (fig. 2S-W) are an example of mostly attached spiral tubes coiled in a certain direction within most genera and species. The most remarkable exception is the problematic *Neomicrorbis* (fig. 2S), having tubes coiled equally in both directions in all species. Among indeterminately coiled tubes, sometimes there are coiling tendencies allowing generic attribution. For example, *Hydroides* species often form wide rounded loops (fig. 2H) and so do fossil *Mucroserpula* and, less often, Recent *Serpula*.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Sculpture (=ornament) and cross-section. Along with coiling mode, external sculpture is the most important character for tube identification. In cases when tubes lack pronounced sculpture (*Apomatus, Hyalopomatus, Protula* - fig. 2C, E), identification of fossils becomes problematic. The tube sculpture typically consists of longitudinal keels (up to 9) or rows of denticles, and transverse ridges and peristomes of varying complexity (fig. 2G, H, J-S, U, W). Keels modify the external cross-section making it (sub)triangular (fig. 2O, P) or multi-angular (fig. 2K, R), and the cross-section is the most robust character allowing generic recognition in fossil state. Transverse peristomes indicate growth stops and can be rare and irregularly spaced (fig. 2L), or almost regularly spaced (e.g. in *Pseudochitinopoma,* fig. 2Q). Sculpture can also be represented by regular pits (e.g. in *Pseudomicrorbis, Metavermilia*, fig. 2K) and alveoli (perforations, fig. 2O), which are usually species-specific rather than characteristic of genera.

Sculpture and tube cross section can change in ontogeny and during the transition to growth away from the substrate. In the latter case cross-section tends to become circular, while longitudinal sculpture disappears and peristomes become more frequent (fig. 2F). Thus, free tube fragments of most genera can hardly be identified with confidence, however, in some taxa (e.g. fossil members of *Vermiliopsis* and *"Filogranula"*) sculpture is well-developed in free fragments as well, and in some taxa (*Pyrgopolon* (*Septenaria*)) keels become even more numerous than in the attached part. Several Recent genera (e.g. *Janita, Pseudochitinopoma, Vitreotubus,* fig. 2R, Q, M, respectively) can be easily recognised by sculpture only; all others show some interspecific variability, however, the limited extent of this variability usually justifies generic attributions.

Internal tube structures. The lumen of serpulid tubes is circular and smooth, but members of genus *Spiraserpula* have unique internal tube structures (ITS), such as longitudinal keels and crests of often fragile appearance inside the lumen (Pillai, 1993; Pillai and ten Hove, 1994; ten Hove and Kupriyanova, 2009). Although *Spiraserpula* seems to be a genus well-recognisable by tube coiling mode, differences in ITS morphology make species recognition a lot easier. Internal tube structures are also known for calcareous sabellids of the genus *Glomerula*, where it was found in some fossil species of Cretaceous-Paleogene age (see Jäger, 1993, 2005; fig. 8A).

Attachment structures. The area of tube attachment is often widened to form basal flanges running along tube sides (e.g. *Pseudovermilia, Spirobranchus*; fig. 2P). When these flanges are continuously hollow (fig. 7H) or subdivided by septae inside (fig. 8P), they are referred to as tubulae (Hedley, 1958: fig. 9; Jäger, 1983: 11, text-fig. 2; Ippolitov, 2007a, b), and probably help the animal to widen and thus to strengthen the attachment area, without requiring too much calcareous material. The frequency of septae inside tubulae has been used as one of justifications for synonymy of the fossil genus *Proliserpula* with Recent *Neovermilia* (Jäger, 1993; 2005).

Tabulae. Some serpulids from clades AI and AII may build inside the tube lumen transverse septae (tabulae) that partition the oldest tube parts as a response to posterior tube damage (ten Hove and Kupriyanova, 2009). Although tabulae are sometimes mentioned by paleontologists (e.g. Müller, 1963; 1970; Nestler, 1963; Ziegler and Michalík, 1980; Ziegler, 1984), their morphology, frequency and variability have not been studied well enough to be useful for classification.

Wall transparency. Tubes of most serpulids can be either opaque or porcellaneous, (i.e. with an internal opaque and external hyaline layer), but *Placostegus, Vitreotubus* (fig. 2N, M, respectively), and some spirorbins (e.g. *Neomicrorbis,* fig. 2S) have completely transparent (hyaline) tubes that can be recognised in the fossilised state. Transparency is determined by certain tube ultrastructure (see below).

Figure 2. Morphological diversity of Recent serpulids. A–R: non-spirorbin serpulids: A – *Ditrupa arietina* (O. F. Müller, 1776), unattached tusk-shaped tubes with circular cross-section. B – *Bathyditrupa hovei* Kupriyanova, 1993a, unattached tusk-shaped tube with quadrangular cross-section (after Kupriyanova et al., 2011: 47, fig. 2E). C – *Apomatus globifer* Théel, 1878, simple tube without sculpture. D – pseudocolony of *Filograna* sp. tubes. E – *Hyalopomatus biformis* (Hartman, 1960), simple tube without sculpture (after Kupriyanova and Nishi, 2010: 62, fig. 5a). F – orange tube of *Serpula vermicularis* Linnaeus, 1758, distal unattached part with peristomes. G – same, attached tube parts with multiple low keels. H – *Hydroides albiceps* (Grube, 1870) tube with flattened upper surface bordered by a pair of keels. I – *Hydroides norvegicus* Gunnerus, 1768, tube without keels, with wavy growth lines. J – *Nogrobs grimaldii* (Fauvel, 1909), unattached spirally coiled tube, quadrangular in cross-section (after Kupriyanova and Nishi, 2011: 2, fig. 1C). K – *Metavermilia arctica* Kupriyanova, 1993b, tube with characteristic combination of transverse and longitudinal sculptural elements resulting in "honey-comb" structure. L – *Ficopomatus enigmaticus* (Fauvel, 1923), aggregation of tubes with irregularly spaced peristomes. M – *Vitreotubus digeronimoi* Zibrowius, 1979, transparent tube with very characteristic flat wide paired keels. N – *Placostegus* sp., transparent tube (after ten Hove and Kupriyanova, 2009: 8, fig. 1F). O – *Spirobranchus polytrema* (Philippi, 1844), tube with single keel and alveoles. P – *Spirobranchus taeniatus* (de Lamarck, 1818), simple tube with single smooth keel and peripheral flanges. Q – *Pseudochitinopoma beneliahuae* Kupriyanova et al., 2012, completely attached tube with transverse ridges (after Kupriyanova et al., 2012: 63, fig. 3A). R – *Janita fimbriata* (delle Chiaje, 1822), tube with very characteristic sculpture. S-W: Spirorbinae: S – *Neomicrorbis azoricus* Zibrowius, 1972, coiled attached tube with numerous keels of denticles (after ten Hove and Kupriyanova, 2009: 65, fig. 29C). T – *Bushiella* (*Bushiella*) *evoluta* (Bush, 1905), clockwise coiled tube with planospiral initial whorls and evoluted distal part. U – *Bushiella* (*Jugaria*) *kofiadii* Rzhavsky, 1988, clockwise coiled tube with distinct keels. V – *Circeis armoricana* de Saint-Joseph, 1894, anticlockwise coiled planospiral tube. W – *Paradexiospira vitrea* (Fabricius, 1780), anticlockwise coiled vitreous tube. A, C, D, F-I, K, L, O, P – photo E. Wong, E, M, Q – photo E. Kupriyanova, B, J – photo E. Nishi, T-W – photo A. Rzhavsky, S – photo R. Bastida-Zavala, R – photo A. Ravara, N – photo G. Rouse. Scale: A – 1 mm, B – 0.5 mm, C – 1 mm, D – 2 mm, E – 0.5 mm, F, G – 5 mm, H, I, J, K – 1 mm, L – 1 mm, M – 2 mm, N-P – 1 mm, Q – 0.5 mm, R – 1 mm, S – 2 mm, T-W – 1 mm.

Opercula. Several serpulid genera (*Spirobranchus*, *Pyrgopolon*, except for fossil subgenus *Pyrgopolon* (*Septenaria*), *Neomicrorbis* and all spirorbins) have fully or partially calcified opercula that fossilise well and are characteristic enough for distinguishing genera and species. Linking fossil tubes and opercula is often problematic as they are usually found separately (but see Cupedo, 1980a, b; Jäger, 2005), resulting even in generic taxa based on opercula only (e.g. Lommerzheim, 1979; 1981). Opercula of *Bathyvermilia*, a Recent genus having thin calcified opercular endplates, are not known in the fossil record. The literature on fossil opercula can be found in full in Radwańska (1994b) and Gatto and Radwańska (2000).

Size. At least two Recent serpulid genera, *Rhodopsis* and *Josephella*, are characterised by minute tubes with diameter not exceeding 0.2 mm, which was used as an argument for attributing minute fossil tubes to *Josephella* (Regenhardt, 1961; Bałuk and Radwański, 1997). In all other genera interspecific variability of tube size is more or less clearly defined, making this character useful for understanding the fossil tube affinity.

All the characters mentioned above are used while determining fossil tubes. Although determination may not be very precise, a combination of characters usually allows making a qualified guess regarding at least the group of closely related Recent genera, "Formenkreis" *sensu* Lommerzheim (1979), where a fossil species belongs. Morphology is used not only for descriptions of fossil species and genera, but also for inferring phylogenetic relationships among those taxa (e.g. Jäger, 1983; 1993; 2005).

In some striking cases taxa originally described by paleontologists by tubes were later found or recognised among Recent serpulids by zoologists. One example of such "living fossils" is the fossil *Neomicrorbis* that was discovered as a bathyal *N. azoricus* Zibrowius, 1972 and recognised by size, coiling, and characteristic sculpture (fig. 2S). Other examples include *Spiraserpula* recognised by ITS found both in fossil and extant taxa (Pillai, 1993; Pillai and ten Hove, 1994) and characteristically coiled calcareous sabellid *Glomerula* known to paleontologists from the early 19th century (Jäger, 2005; Ippolitov, 2007a), but discovered in Recent fauna only recently (Perkins, 1991). Recent *Spirodiscus* (fig. 2J) with distinct spirally coiled quadrangular tubes was synonymised with fossil genus *Nogrobs* (Jäger, 2005; ten Hove and Kupriyanova, 2009) having very similar tubes, and Recent *Sclerostyla* was considered a synonym of fossil *Pyrgopolon* (Jäger, 1993; 2005) based on tube shape, size, sculpture, and very characteristic calcified opercula (Wrigley, 1951; Cupedo, 1980a, b).

2.3. Tube ultrastructures: a new tool in serpulid systematics?

Studies over the last three decades revealed extensive ultrastructural diversity in serpulid tube walls (e.g. Bohnné Havas, 1981; Bubel et al., 1983; Bandel, 1986; ten Hove and Zibrowius, 1986; Zibrowius and ten Hove, 1987; Nishi, 1993; Sanfilippo, 1998a, b; 2001; Vinn, 2005; 2007; 2008; Vinn et al., 2008b, d). Vinn et al. (2008b) recognised four main groups of tube ultrastructures in serpulids according to orientation of calcium carbonate crystals: 1) isotropic structures (the crystallisation axis lacks a uniform orientation, fig. 3A-E); 2) semi-oriented structures (the crystallisation axis has semi-uniform orientation, fig. 3F, G); 3) oriented prismatic structures (the crystallisation axis has a uniform orientation and is continuous through successive growth increments, fig. 3H, I, M-O); and 4) oriented complex structures (the crystallisation axis of the crystals has a uniform orientation that is not continuous through successive growth increments, fig. 3J-L). In total, 13 distinct ultrastructures (Vinn et al., 2008b, d) are currently recognised in Recent serpulids (fig. 3, 4).

These 13 types can be arranged into several (up to 4) tube layers, though the majority of species have single-layered tubes. Vinn et al. (2008b) examined 44 species belonging to 36 genera and showed that 47% of studied species possess a unique combination (ultrastructural types and their arrangement into layers) of tube characters. Most advanced and highly ordered types of structures are difficult to explain from the point of the classic for serpulids "granular secreting" model (Neff, 1971), so a matrix-mediated model of biomineralisation was proposed (Vinn et al., 2009).

Ultrastructures of Recent tubes may show inter-specific variability (Vinn, 2007; Ippolitov and Rzhavsky, 2008) and can even have a more or less clear adaptive significance (Sanfilippo, 1996; Vinn and Kupriyanova, 2011), but intra-generic variability of ultrastructures is poorly understood. The idea that generic affiliation of fossils can be evaluated using tube ultrastructures was first proposed by Sanfilippo (1998b). The ultrastructural investigation into fossil tubes has recently commenced (e.g. Sanfilippo, 1998a; 1999; Vinn, 2005; 2007; 2008; Vinn and Furrer, 2008; Vinn et al., 2012) and has already helped to prove the serpulid nature of fossils in some doubtful cases (Vinn et al., 2008c; Taylor, 2014).

Ultrastructures can potentially be used to distinguish serpulid taxa and even to verify linking fossils with recent taxa (Kupriyanova and Ippolitov, 2012) and thus, they may be crucially important for further interpretation of the fossil record and understanding serpulid evolution. However, the ultrastructural method is not widely used to estimate the systematic position of Recent and fossil tubes for two reasons. First, ultrastructural variability within Recent genera is insufficiently studied for any meaningful comparison with fossils. Second, fossil material is often diagenetically altered (i.e. original mineralogy, crystal shapes and arrangement may be changed during the sediment to rock transformation); although direct comparisons are still possible, they are restricted to well-preserved fossil material (fig. 5D-I).

Comparison of ultrastructural variation with molecular phylogenies (e.g. Kupriyanova and Nishi, 2010) reveals a striking difference in the complexity of tube ultrastructures between the two major clades. The complex oriented structures and the oriented prismatic structures restricted to the clade A (Vinn et al., 2008b; Vinn and Kupriyanova, 2011: fig. 1) seem to be derived from isotropic structures that are considered to be plesiomorphic (Vinn, 2013c). However, oriented prismatic structures are also known for spirorbins (Ippolitov and Rzhavsky, 2008) nested inside clade B that predominantly has isotropic structures, thus suggesting an evolutionarily independent origin. In both clade A (Vinn and Kupriyanova, 2011) and in spirorbins (Ippolitov and Rzhavsky, 2008)

Table 2. Main “fossil” serpulid genera still not recognised in Recent fauna. Uncommon genera, taxa of doubtful validity, and taxa erroneously described as serpulids (e.g. numerous Paleozoic genera listed in Ziegler, 2006) are not included. For designations see Table 1.

Fossil genera and most common synonyms	**Number of species**	**Known stratigraphic range**	**Comments**
NON-SPIRORBIN SERPULIDS			
†*Austrorotularia* Macellari, 1984	8	Kimmeridgian to Maastrichtian (157-66 Ma)	originally described as a subgenus of †*Rotularia*, but likely a separate lineage
†*Cementula* Brünnich Nielsen, 1931	10+	?Late Pliensbachian to ?Late Burdigalian (184-17 Ma)	included species partly may be related to *Serpula*/*Hydroides*, partly to *Spiraserpula* with reduced ITS, and partly to sabellid *Glomerula*. In paleontological literature also as subgenus of *Serpula* (see Jäger and Schneider, 2009).
†*Conorca* Regenhardt, 1961	5	?Cenomanian, Turonian to Maastrichtian (?100, 92-66 Ma)	
†*Corynotrypoides* Bizzarini et Braga, 1994	1	Carnian (237-227 Ma)	originally described as cyclostome bryozoan, serpulid affinities proposed by Taylor (2014)
†*Cycloplacostegus* Jäger, 2005	2	?Late Turonian, Early Santonian to Early Maastrichtian (?91, 86-71 Ma)	
†*Dorsoserpula* Parsch, 1956	6+	Middle Oxfordian to latest Maastrichian (160-66 Ma)	
†*Genicularia* Quenstedt, 1856	1+	Early Oxfordian (163 Ma)	
†*Jereminella* Lugeon, 1919	1	Maastrichtian (72-66 Ma)	doubtful validity, poorly studied genus
†*Laqueoserpula* Lommerzheim, 1979	5+	Late Oxfordian to latest Maastrichtian (159-66 Ma)	doubtful status, may be related to *Filogranula, Metavermilia* or other genera
†*Martina* Ziegler, 1984	1+	Early Turonian (93 Ma; Ziegler, 1984)	*nomen dubium*
†*Mucroserpula* Regenhardt, 1961	6+	?Late Pliensbachian (Jäger and Schubert, 2008); Bajocian to Maastrichtian (?184, 170-66 Ma)	large-sized representatives from the Pliensbachian may belong to †*Propomatoceros*
†*Octogonella* Ziegler, 2006	1	Middle Danian (64 Ma)	doubtful validity, may be a synonym of *Pyrgopolon*
†*Orthoconorca* Jäger, 1983	7+	Late Albian to Late Danian (~105-~62 Ma)	
†*Paliurus* Gabb, 1876	2	Cenomanian to Eocene (100-34 Ma)	doubtful validity, revision needed
†*Pannoserpula* Jäger et al., 2001	3	Middle Oxfordian to Late Kimmeridgian (161-152 Ma)	
†*Parsimonia* Regenhardt, 1961	5+	Late Volgian to Middle Santonian, ?Campanian to Maastrichian (~147-85 Ma, ?80-66 Ma)	partly may be a synonym of *Serpula*
†*Pentaditrupa* Regenhardt, 1961	4+	Hettangian to Danian (201-62 Ma; Jäger 2005)	may be a synonym or subgenus of †*Genicularia*

Fossil genera and most common synonyms	Number of species	Known stratigraphic range	Comments
†*Propomatoceros* Ware, 1975	24+	Pliensbachian to Turonian (190-~89 Ma)	some species included in the genus may be referred to *Serpula* and *Spirobranchus*. Upper time limit is uncertain, as Cretaceous species listed by Ippolitov (2007b) need revision
†*Protectoconorca* Jäger, 1983	2	Cenomanian to Maastrichtian (100-66 Ma)	
†*Rotularia* Defrance, 1827a =†*Spirulaea* Bronn, 1827	20+	Danian to Priabonian (66-34 Ma)	all subgenera, classically treated under this genus (e.g., Regenhardt, 1961; Jäger, 1993) are considered as separate genera in the present paper
†*Rotulispira* Chiplonkar et Tapaswi, 1973b =†*Praerotularia* Lommerzheim, 1979	20+	Hauterivian to ?Maastrichtian (133-?66 Ma)	
†*Ruxingella* Stiller, 2000	1	Late Anisian (244 Ma)	questionable validity, as no comparison with other fossil and Recent taxa provided
†*Sarcinella* Regenhardt, 1961	1	Middle Jurassic to Early Campanian (~174-80 Ma; Jäger, 2005)	
†*Tectorotularia* Regenhardt, 1961	10+	Hauterivian to Maastichtian (133-66 Ma)	doubtful validity, partly (including type species) may belong to †*Tubulostium* Stoliczka, 1868. Originally †*Tectorotularia* was described as a subgenus of †*Rotularia*, but likely a separate lineage
†*Triditrupa* Regenhardt, 1961	1	Cenomanian (100-94 Ma)	originally described as a subgenus of *Ditrupa*, but likely a separate lineage. Doubtful status, maybe a subgenus of *Pyrgopolon* (Jäger, 1993, 2005).
†*Tubulostium* Stoliczka, 1868 ?=†*Tectorotularia* Regenhardt, 1961	2	Albian to Turonian (113-90 Ma)	doubtful validity, may be a synonym of *Nogrobs* de Montfort, 1808 (*s. str.*)
†*Weixiserpula* Stiller, 2000	1	Late Anisian (244 Ma)	questionable validity, as no comparison with other fossil and Recent taxa provided
SPIRORBINAE			
†*Bipygmaeus* Regenhardt, 1961	2	Early Cenomanian to Middle Danian (100-63 Ma)	
†*Cubiculovinea* Lommerzheim, 1981	1	Middle Paleocene (62-59 Ma)	genus description based on opercula only
†*Ornatovinea* Lommerzheim, 1979	1	Earliest Cenomanian (~100 Ma)	genus description based on opercula only
DOUBTFUL SPIRORBINAE			
†*Pseudomicrorbis* Jäger, 2011	1	Late Berriasian to Barremian (~142-~125 Ma)	

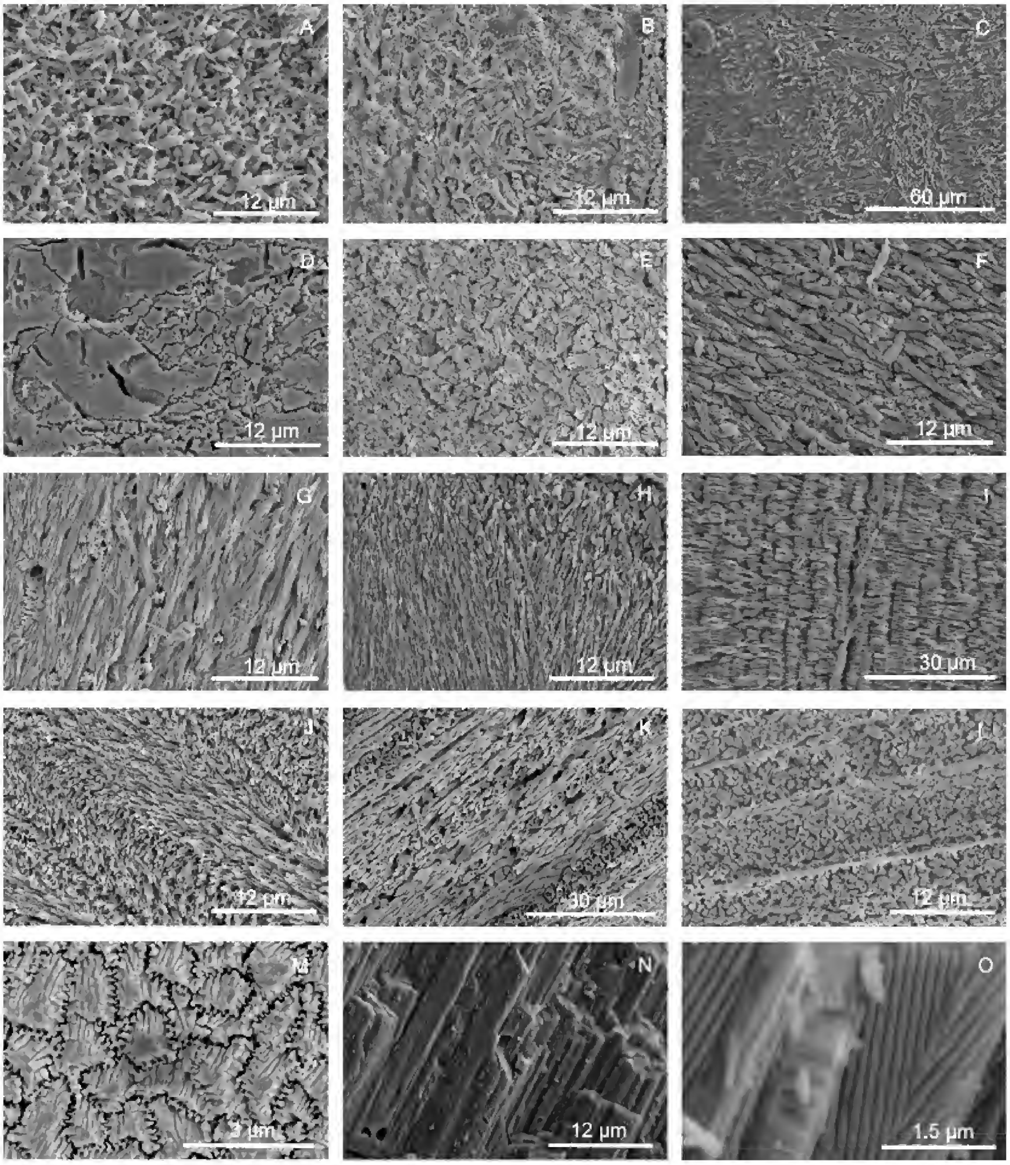


Figure 3. Ultrastructural diversity of Recent serpulid tubes. A-E: isotropic structures: A – *Serpula crenata* Ehlers, 1908, inner tube layer, cross section of irregularly oriented prismatic structure (IOP), B – *Pseudovermilia madracicola* ten Hove, 1989, cross section of spherulitic irregularly oriented prismatic structure (SIOP) (after Vinn et al., 2008b: fig. 2A), C – *Neovermilia falcigera* (Roule, 1898), cross section of irregularly oriented platy structure (IOPL), D – *Laminatubus alvini* ten Hove et Zibrowius, 1986, cross section of homogeneous angular crystal structure (HAC), E – *Pomatostegus stellatus* (Abildgaard, 1789), cross section of homogeneous rounded crystal structure (HRC) (after Vinn et al., 2008b: fig. 3E), F, G: semi-oriented structures: F – *Protula diomedeae* Benedict, 1887, cross section of semi-ordered irregularly oriented prismatic structure (SOIOP) (after Vinn, 2007: fig. 5.5), G – *Pyrgopolon ctenactis* Mörch, 1863, outer tube layer, cross section of semi-ordered spherulitic irregularly oriented prismatic structure (SOSIOP) (after Vinn, 2007: fig. 7.4), H, I and M-O: oriented prismatic structures: H – *Spiraserpula caribensis* Pillai et ten Hove, 1994, outer tube layer, longitudinal section of spherulitic prismatic structure (SPHP) (after Vinn, 2007: fig. 6.5), I – *Vitreotubus digeronimoi* Zibrowius, 1979, longitudinal section of simple prismatic structure (SP) (after Vinn et al., 2008b: fig. 5B, enlarged), J-L: oriented complex structures: J – *Hydroides dianthus* Verrill, 1873, third layer from outside, longitudinal section of lamello-fibrillar structure (LF) (after Vinn, 2008: fig. 4.5), K – *Floriprotis sabiuraensis* Uchida, 1978, inner layer, cross section of spherulitic lamello-fibrillar structure (SLF), L – *Spirobranchus giganteus* (Pallas, 1766), outer layer, longitudinal section of ordered fibrillar structure (OF) (after Vinn et al., 2008b: fig. 6B), M-O – *Ditrupa arietina* (O. F. Müller, 1776), regularly ridged prismatic structure (RRP): M – tube external surface, etched with 1% acetic acid for 30 sec (after Vinn et al., 2008d: fig. 3F), N – external tube layer, longitudinal section, O – lateral surface of a RRP structure prism with ridges (after Vinn et al., 2008d: fig. 4A).

oriented prismatic structures tend to form dense outer tube layer near the surface of the wall. Unilayered tubes with prismatic structure of the only layer are transparent (Ippolitov and Rzhavsky, 2008; Vinn et al., 2008b) because of parallel orientation of optical axes in crystals.

2.4. Tube mineral composition: new cues for serpulid evolution

Tubes of serpulids consist of calcite, aragonite, or a mixture of both modifications of calcium carbonate (Bornhold and Milliman, 1973; Vinn et al., 2008b) interspersed with an organic mucopolysaccharide matrix. The first comprehensive overview of serpulid tube mineralogy by Bornhold and Milliman (1973) provides data for over 100 specimens belonging to 24 species of 11 genera. The study found only limited correlations of tube mineralogical composition with environmental factors and with classification. However, data on mineralogical composition have been used to test the generic affiliation of serpulid tubes (Ferrero et al., 2005) and to distinguish species within a single genus (e.g. Bornhold and Milliman, 1973; followed by ten Hove, 1974: 47).

Calcite and aragonite are rarely present in almost equal quantities within one tube, and calcite-aragonite ratio may significantly vary not only among species, but also within a species and even within a single tube during the ontogeny (Bornhold and Milliman, 1973). Vinn et al. (2008b) found some correlations between mineralogy and ultrastructural types, showing that lamello-fibrillar ultrastructure, mainly known for clade A, is exclusively calcitic. Similarly, the simple prismatic ultrastructural type is clearly correlated with calcite mineralogy.

When mapped to existing phylogeny, aragonitic mineralogy is predominantly associated with the “filogranin” non-spirorbin clade BI having simple un-oriented structures, while calcitic mineralogy is more typical for clade A showing complex ultrastructures (Vinn, 2012). Aragonitic irregularly oriented prismatic structure (fig. 3A, 4A) appears to be plesiomorphic for serpulids (Vinn and Kupriyanova, 2011), while complex oriented calcitic structures are far more advanced. Vinn (2012) hypothesised that calcite is favoured by the serpulid biomineralisation system for producing complex structures. In contrast, within molluscs aragonite has a greater variety of complex structures as compared to that of calcite (Carter et al., 1990). Recently Smith et al. (2013) also showed that clade AI (“*Serpula*-group”) tends to have mixed mineralogy with dominating calcite, and clade AII (“*Spirobranchus*-group”) tends to have fully calcitic mineralogy, sometimes with little aragonite. Again no clear correlations with environmental factors were found.

According to the hypothesis by Vinn and Mutvei (2009), supported by Smith et al. (2013), ocean chemistry was the dominant factor controlling the evolution of serpulid tube mineralogy over geological time periods with differing conditions favouring the precipitation of a certain mineral (so-called ”calcitic” and “aragonitic” seas, see Stanley, 2006). According to this idea, plesiomorphic serpulids of clade BI tend to have aragonitic mineralogy because they originated and diverged in aragonitic seas of the Triassic period, while more advanced calcitic serpulids of clade A mainly evolved during the Jurassic-Cretaceous time, which was the epoch of calcitic seas.

2.5. Organic component of tubes: will biochemistry meet paleontology?

The only approach complementing ultrastructural and mineralogical studies is histochemical investigation of the organic tube component as suggested by ten Hove and van den Hurk (1993) and Gatto and Radwańska (2000). This organic component is represented by an inner organic membrane lining the lumen and an organic matrix inside the tube walls. The inner organic membrane is found in all serpulids (Nishi, 1993; Vinn, 2011) and may play an important role for the biomineralisation process (Tanur et al., 2010), but this needs further clarification (Vinn, 2011). The organic matrix of the tube wall should be preserved in fossil serpulid tubes, as it does in mollusc shells. The tube matrix seems to be organised in thin sheets running parallel to accretion surfaces (Vinn et al., 2008b), but such organisation was observed only in some taxa within clade A (Vinn, 2013b). Tanur et al. (2010) found that most of the soluble organic tube matrix of a Recent species *Hydroides dianthus* (Verill, 1873) is composed of carboxylated and sulfated polysaccharides, whereas proteins form a minor component. No data on other species are available and further studies are needed to determine usability and potential of this method for paleontology.

3. An outline of serpulid evolution as revealed by fossils

3.1. False serpulids: tubular fossils below the Precambrian-Cambrian boundary (~541 Ma)

During so-called “Cambrian explosion”, an episode in the Earth history that took place about 541 Ma, most major fossil invertebrate groups suddenly appeared in paleontological record within a short time interval, often having developed mineral structures within or around the body.

Many tubular fossils of problematic affinity appeared already during the preceding Late Ediacaran (~577-541 Ma). They include chitinous tubes of sabelliditids, often considered to be the ancestors of Recent Siboglinidae, and calcified tubular problematics *Cloudina*, *Sinotubulite*s (Chen et al., 2008), as well as unusual forms with triradial symmetry, such as *Anabarites* (Kouchinsky et al., 2009). Many of these tubular fossils have been attributed to annelids in general and serpulids in particular (e.g. Yochelson, 1971; Glaessner, 1976; Chen et al., 1981; Bandel, 1986), but their true biological affinities are usually unresolved. The major function of mineralised tubes was probably protection against predation (Bengston, 2002), but physiological adaptation to changing ocean chemistry and the opportunity to grow larger were also proposed (e.g. Bengston, 2004: 69-70).

Cloudina (fig. 6A), the most famous tube-building metazoan common in deposits of the terminal Neoproterozoic Ediacaran Period (549-541 Ma), has often been affiliated with serpulids (Germs, 1972; Glaessner, 1976; Hua et al., 2005). Tube morphology and microgranular ultrastructure (fig. 5A) suggest that *Cloudina* is not closely related to any Recent calcareous polychaetes (serpulids, sabellids or cirratulids) (Vinn and Zatoń, 2012a). The type of asexual reproduction and presence of a closed tube base in *Cloudina* is more compatible

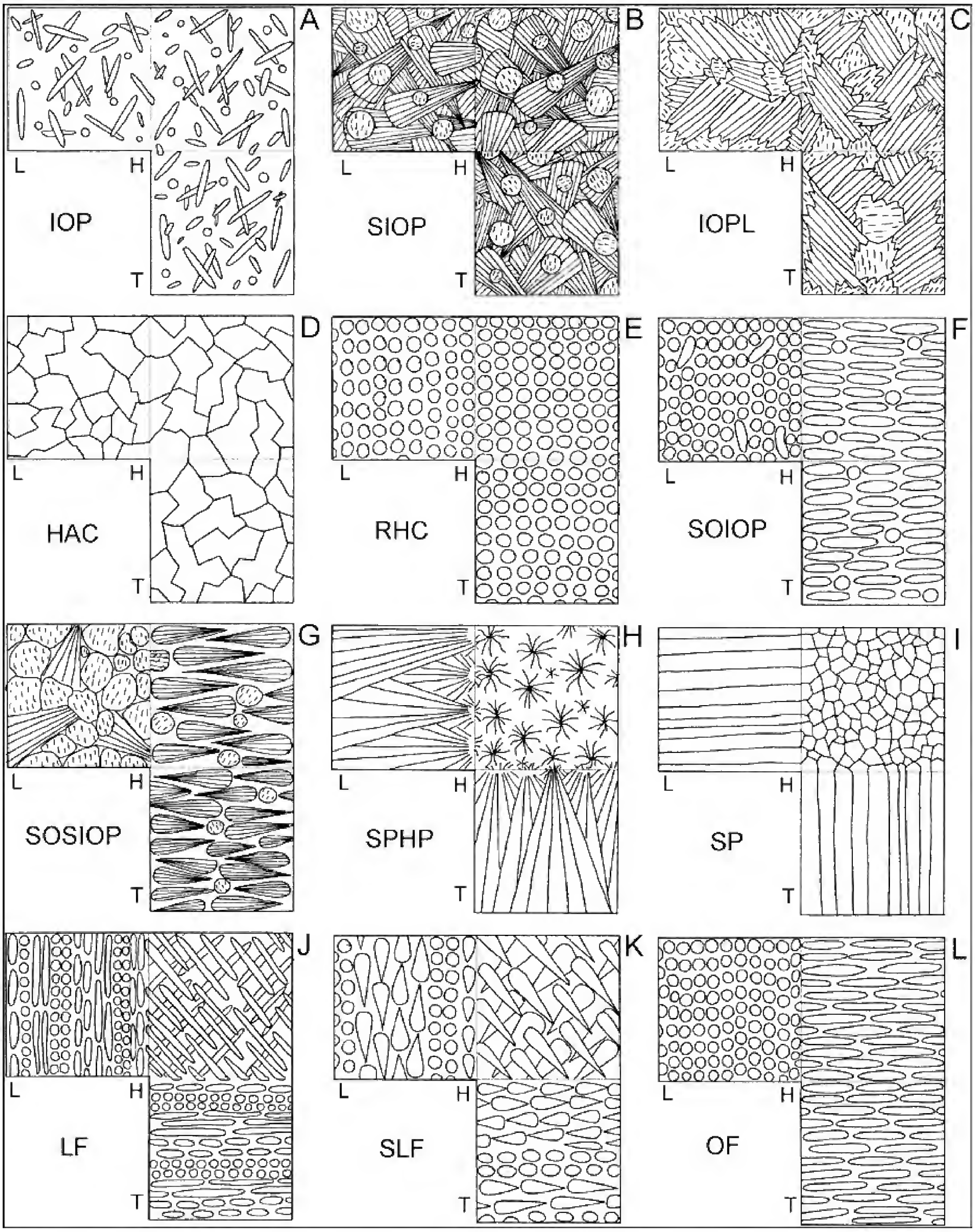


Figure 4. Schematic presentation of serpulid tube ultrastructures (from Vinn et al., 2008b). A – irregularly oriented prismatic (IOP) structure. B – spherulitic irregularly oriented prismatic (SIOP) structure. C – irregularly oriented platy (IOPL) structure. D – homogeneous angular crystal (HAC) structure. E – rounded homogeneous crystal (RHC) structure. F – semi-ordered irregularly oriented prismatic (SOIOP) structure. G – semi-ordered spherulitic irregularly oriented prismatic (SOSIOP) structure. H – spherulitic prismatic (SPHP) structure. I – simple prismatic (SP) structure. J – lamello-fibrillar (LF) structure. K – spherulitic lamello-fibrillar (SLF) structure. L – ordered fibrillar (OF) structure. Regularly ridged prismatic structure (RRP, see fig. 3 M-O) is similar to SP structure. Abbreviations: H: horizontal section; L: longitudinal section; T: transverse section.

with the hypothesis of an animal of cnidarian grade (Hua et al., 2005; Vinn and Zatoń, 2012a; Zhuravlev et al., 2012).

3.2. Paleozoic (541-252 Ma) tubular problematic taxa

Paleozoic rocks, especially of Early Cambrian age (~540 Ma), contain tubular fossils of uncertain affinities, some of which are carbonate (e.g. *Coleolella*), and others are phosphatic (*Hyolithellus, Sphenothallus*) or even siliceous (*Platysolenites*). Among Paleozoic fossils, two common and diverse fossil groups, Cornulitida and Microconchida, have traditionally been described as serpulids. Including them in the serpulid fossil record resulted in a long-held controversy regarding the geological age of calcareous polychaetes and in wrong interpretations of evolutionary patterns within the Serpulidae by both zoologists (e.g. Pillai, 1970; Knight-Jones, 1981) and paleontologists (Jäger, 1993: 101).

Cornulitids (fig. 6B) are mostly small (2-5 mm, although some species could reach 25 mm in tube diameter) calcareous tubular fossils ranging from the Middle Ordovician to the Carboniferous (470-300 Ma) and found in normal marine settings. They have been affiliated with annelids due to the tubular shape of their shells. Similar to modern serpulids, cornulitids were presumably suspension feeders and common encrusters on Paleozoic hard substrates. Their biological affinities have long been debated, but they could represent stem group of phoronids (Taylor et al., 2010). Recent analysis by Vinn and Zatoń (2012b) places them with confidence within the Lophotrochozoa.

Microconchids (fig. 6C) are a *Spirorbis*-like extinct group of lophophorates ranging from the Late Ordovician to the Middle Jurassic (458-164 Ma) that inhabited all aquatic environments from hypersaline to freshwater (Zatoń et al., 2012). Due to their small size (usually <1 mm, up to 2 mm in coil diameter) and obligatory spirally coiled calcareous tubes, for decades microconchids were treated as spirorbins (e.g. Goldfuss, 1831; Zittel, 1880; Malaquin, 1904; Howell, 1962;

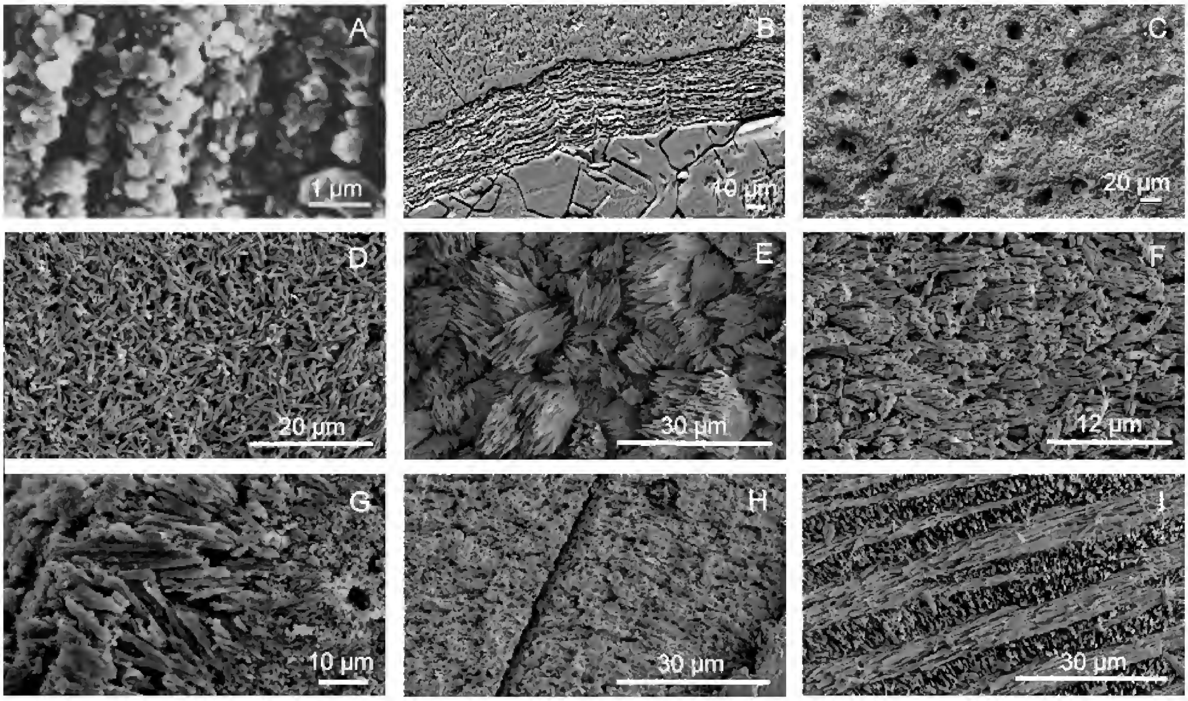


Figure 5. Ultrastructural diversity of fossil serpulids and some typical "pseudoserpulids". A-C: ultrastructures of most characteristic pseudoserpulids: A – *Cloudina sinensis* Zhang et al. in Ding et al., 1992, showing microgranular structure; Late Ediacaran (549-542 Ma), China (after Feng et al., 2003: fig. 1b). B – microconchoid *Palaeoconchus tenuis* (Sowerby in Murchison, 1839), Silurian (Wenlockian; 433-427 Ma), England (after Vinn, 2006: fig. 4). C – microconchoid *Punctaconchus ampliporus* Vinn et Taylor, 2007, surface showing pores; Middle Jurassic (Bathonian, 168-166 Ma), U.K. (after Vinn and Taylor, 2007: fig. A_2). D-I: ultrastructures of fossil serpulids: D – '*Serpula*' *etalensis* (Piette, 1856), longitudinal section of irregularly oriented prismatic structure (IOP); Early Jurassic, Late Pliensbachian (~185 Ma), eastern Germany (after Vinn et al., 2008c: fig. 1D). E – *Rotularia spirulaea* (Lamarck, 1818), longitudinal section of homogeneous angular crystal structure? (HAC); Eocene (56-34 Ma) of Doss Trento, Northern Italy. F – *Protula* sp., cross section of semi-ordered irregularly oriented prismatic structure (SOIOP); Tongrian, Late Eocene (~35 Ma), Latdorf, North Germany (after Vinn, 2007: fig. 3.1, detail). G – *Propomatoceros* sp., outer tube layer, spherulitic prismatic structure (SPHP); Middle Volgian (~148 Ma), Samara region, Russia. H – *Placostegus polymorphus* Rovereto, 1895, cross section of simple prismatic structure (SP); Badenian (~15 Ma), Miocene, Ehrenhausen, Styria, Austria (after Vinn, 2007: fig. 1.5, detail). I – *Spiraserpula* sp., oblique section of lamello-fibrillar structure (LF); Badenian (~15 Ma), Miocene, Nussdorf, Vienna, Austria (after Vinn, 2007: fig. 4.5).

Regenhardt, 1964; Pillai, 1970; Lommerzheim, 1979; 1981; Jäger, 1983; 1993). Burchette and Riding (1977) who analysed microconchid morphology and tube ultrastructure, were the first to justify doubts about their annelid affinities and interpreted them as gastropods. The microconchid microlamellar tube wall (fig. 5B) with small pores (fig. 5C) is incompatible with known serpulid ultrastructural diversity, and currently microconchids are interpreted as extinct tentaculitoids (Weedon, 1991; Taylor and Vinn, 2006).

None of the reports of Paleozoic serpulids, starting from Cambrian and Ordovician (e.g. Dalvé, 1948; Clausen and Álvaro, 2002) and continued by Devonian (e.g. Sandberger and Sandberger, 1856) records, show the presence of unequivocal serpulid tube characters (such as, for example, a

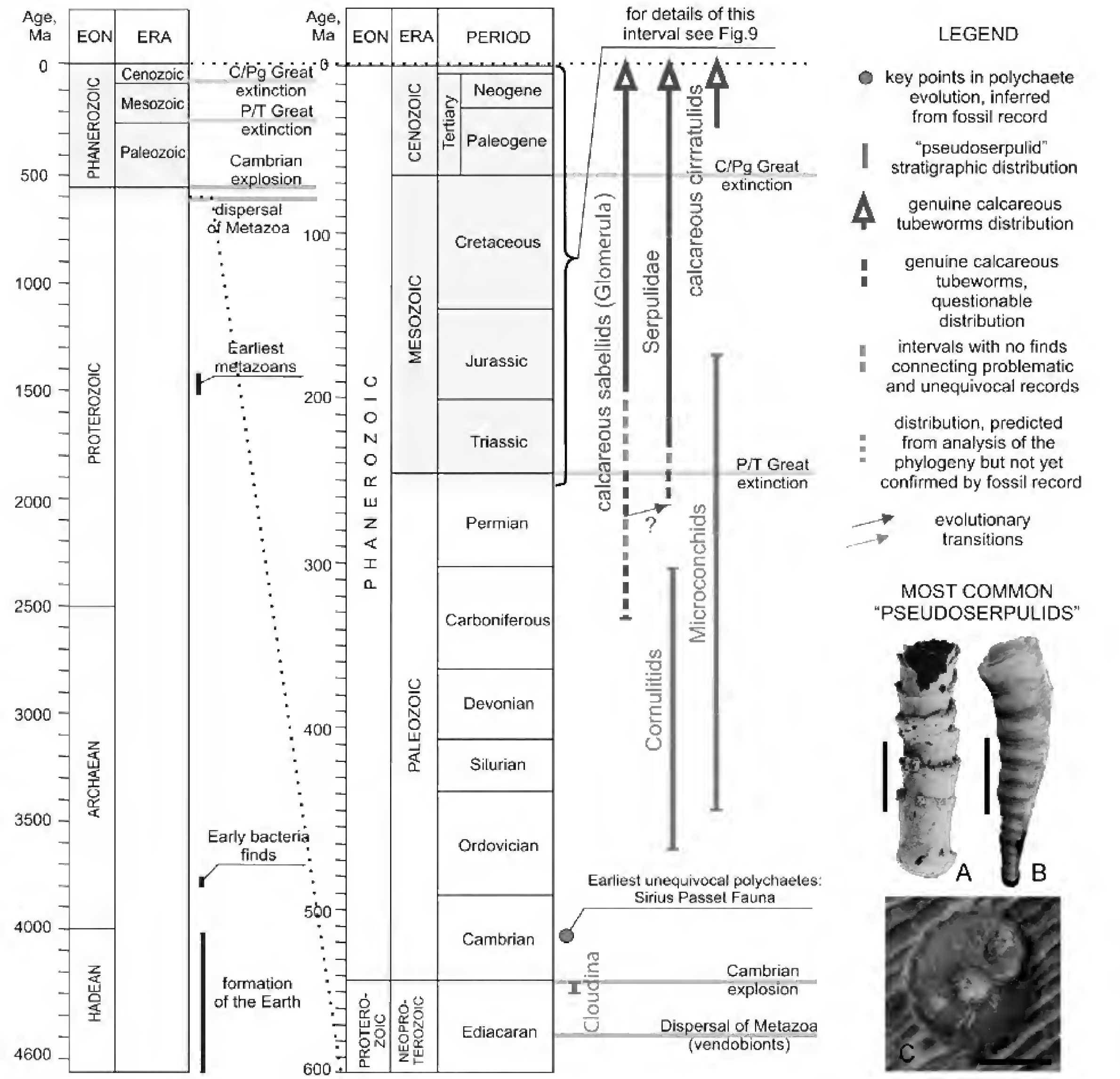


Figure 6. Outline of geological history of calcareous polychaetes and some convergent tube-dwelling taxa ("pseudoserpulids") during the Phanerozoic. A – *Cloudina hartmannae* Germs, 1972, SEM, Late Ediacaran (549-542 Ma), China (after Hua et al., 2005: fig. 1A). B – *Cornulites* sp., Early Ordovician (485-470 Ma), Estonia (after Vinn, 2013a: fig. 8). C – microconchoid *Palaeoconchus tenuis* (Sowerby in Murchison, 1839), Silurian (Wenlockian; 433-427 Ma), England (after Vinn, 2006: fig. 4). Scale: A – 3 mm, B – 0.5 mm, C – 1 mm.

median keel or tubules). Many of these finds still should be re-investigated to check their annelid affinity. The most confusing records of numerous Paleozoic serpulid genera are provided in the overview by Ziegler (2006), who treated almost all existing tubular fossils as serpulids. There is no reason to support such an opinion.

3.3. Possible calcareous tubeworms of the Late Paleozoic

Some Late Carboniferous to Permian records of calcareous tubes likely belong to the sabellid genus *Glomerula* judging by their slowly growing tubes with characteristic glomerate coiling. The most ancient among them are the Late Carboniferous (323-304 Ma) "tubeworms" (Hoare et al., 2002, fig. 1.1-1.7) and probably also species described as "*Serpula*" spp. by Stuckenberg (1905). Younger finds of the same type are Late Permian (265-254 Ma) fossils from Australia described as *Serpula testatrix* Etheridge, 1892. All these finds are characterised by the tube diameter of only about 0.25 mm, while younger Mesozoic *Glomerula* tubes (fig. 7C-E) can reach up to 4-5 mm in diameter, and tubes of the only known Recent species *G. piloseta* (Perkins, 1991) have diameters about 0.5 mm. Sabellids seem to have a primitive biomineralisation system compared to that of serpulids (Vinn and Mutvei, 2009), and thus their earlier representatives may be interpreted as common ancestors of calcified sabellids and serpulids.

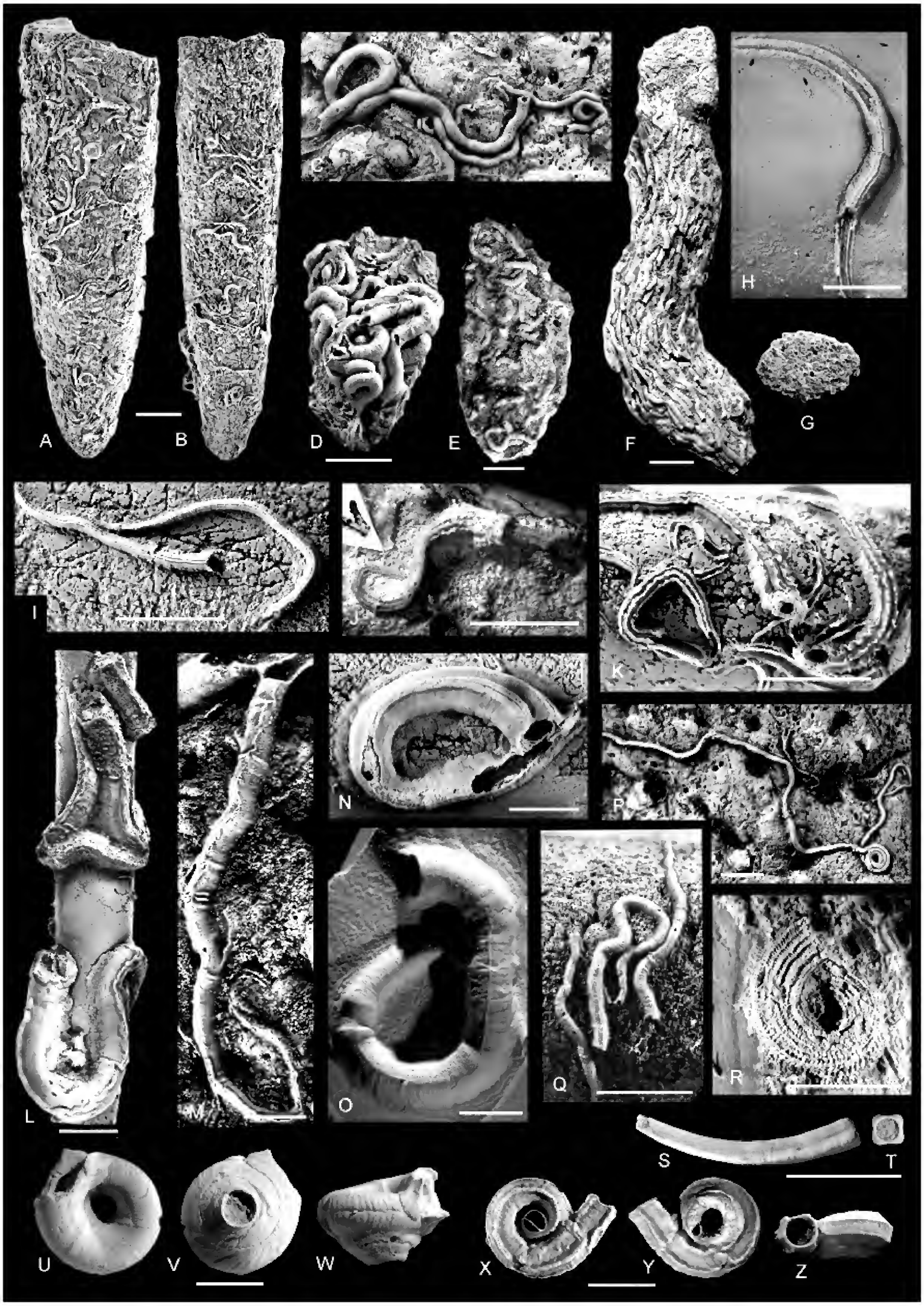
More or less coeval are Late Permian finds of attached tubes that do not show typical glomerate coiling and, therefore, may potentially represent true serpulids (e.g. some figured specimens of "*Serpula pusilla* Geinitz, 1848", "*Vermilia*" *obscura* King, 1850 and maybe "*Serpulites*" from Australia (Guppy et al., 1951)). Such fossils were also reported from Lithuania by Suveizdis (1963). Due to small size of these fossils, similar to that of above-mentioned sabellids, details of their morphology are unclear from old descriptions and figures, so their potentially serpulid nature is yet to be re-investigated.

3.4. Earliest records of genuine serpulids

Serpulids seem to rise soon after the Permian-Triassic boundary, famous for being the largest extinction event in geological history. Adequately preserved fossils of first unequivocal serpulids from the Middle Triassic (Late Anisian, ~244 Ma) of China are represented by strange tiny tubes lacking any sculpture or having an indistinct single median keel. They were described within two new genera as *Weixiserpula weixi* Stiller, 2000 and *Ruxingella lianjiangensis* Stiller, 2000. Exactly of the same age (Late Anisian; ~244 Ma) are the first unequivocal finds of small pseudocolonial tubes described as *Filograna minor* by Senowbary-Daryan et al. (2007) from Turkey, and a diversified community described by Assmann (1937) from Upper Silesia (Western Poland). The latter, besides *Filograna* morphotype, includes large-sized tubes, some of which have longitudinal sculpture and some show a tendency to build aggregations. Slightly younger (Ladinian; ~242-237 Ma) are records of tubes from South Europe with more or less prominent single median keels (Flügel et al., 1984: 186, Pl. 26, fig. 9).

During the Late Triassic serpulids became widely distributed along the northern and southern margins of the Tethys Ocean. Fossil tubes morphologically similar to Recent morphotypes are known from Indonesia (Jaworski, 1915) and Europe (Münster, 1841; Ziegler and Michalík, 1980; Jadoul et al., 2005, fig. 4c). Some of them are large-sized forms, with tube diameters up to 5-6 mm, but mostly unsculptured. Numerous records of small tube bundles from the Late Triassic sediments of Southern Europe and Turkey (Schmidt and von Pia, 1935; Senowbari-Daryan and Link, 2005) comparable to those of Recent *Filograna* (fig. 2D, 7F-G) indicate wide dispersal of this genus during the

Figure 7. Morphological diversity of Jurassic and Cretaceous (mainly Early Cretaceous) tube-dwelling polychaetes. A, B – fossil serpulid communities encrusting belemnite rostra, PIN 5071/100 and 5071/101, respectively; Middle Oxfordian (161 Ma), Kostroma region, Russia. C-E: calcareous sabellids: C – *Glomerula flaccida* (Goldfuss, 1831), PIN 5071/2, Late Callovian (163.5 Ma), Moscow region, Russia (after Ippolitov, 2007a: Pl. 7, fig. 2); D – *Glomerula gordialis* (von Schlotheim, 1820) with characteristic glomerate coiling, PIN 5071/102, Middle or Late Oxfordian (161-158 Ma), Mordovia region, Russia; E – *Glomerula* cf. *plexus* (J. de C. Sowerby, 1829), pseudocolonial form, PIN 5071/106; Middle Volgian (150 Ma), Samara region, Russia. F-J: possible members of serpulid clade BI: F-G – *Filograna socialis* (Goldfuss, 1831), pseudocolonial form, PIN 5071/109; Middle Volgian (150 Ma), Orenburg region, Russia; H – *Metavermilia goldfussi* Ippolitov, 2007a, PIN 5071/15, Late Callovian (163.5 Ma), Moscow region, Russia (after Ippolitov, 2007a: Pl. 7, fig.15); I – *Metavermilia striatissima* (Fürsich, Palmer et Goodyear, 1994), PIN 5071/134(1, 2), Late Oxfordian (159 Ma), Kostroma region, Russia; J – *Vermiliopsis negevensis* Vinn et Wilson, 2010, TUG 1372-2, Late Callovian (~164 Ma), Israel (after Vinn and Wilson, 2010: fig. 6.2). K-O – possible members of serpulid clade AII: K – "*Filogranula*" *runcinata* (J. de C. Sowerby, 1829), PIN 5071/112(1, 2), Middle Oxfordian (161 Ma), Kostroma region, Russia; L – *Propomatoceros lumbricalis* (von Schlotheim, 1820), No. 5071/24-28, Late Callovian (163.5 Ma), Moscow region, Russia (after Ippolitov, 2007b: Pl. 12, fig. 3); M – the same, PIN 5071/36, same age and locality (after Ippolitov, 2007b: Pl. 12, fig. 7); N – *Mucroserpula tricarinata* (J. de C. Sowerby, 1829), PIN 5071/19, Late Callovian (163.5 Ma), Moscow region, Russia (after Ippolitov, 2007b: Pl. 12, fig. 2); O – *Neovermilia ampullacea* (J. de C. Sowerby, 1829), PIN 5204/9, ?Turonian (94-89 Ma), Bryansk region, Russia. P-Q: probable members of serpulid clade AI: P – *Spiraserpula oligospiralis* Ippolitov, 2007b, PIN 5071/50 (holotype), Late Callovian (163.5 Ma), Moscow region, Russia (after Ippolitov, 2007b: Pl. 12, fig. 11); Q – "*Serpula*" sp. nov., PIN 5071/136 (1, 2, 3), Late Oxfordian (~158 Ma), Kostroma region, Russia. R-Z: clade uncertain: R – *Pseudomicrorbis* cf. *pseudomicrorbis* Jäger, 2011, problematic taxon interpreted as close to plesiomorphic spirorbins, PIN 5071/150, Late Berriassian (~141 Ma), Crimea, Ukraine; S-T: *Nogrobs* (*Tetraserpula*) *barremicus* (Sasonova, 1958), PIN 5071/151, Late Barremian (~126 Ma), Saratov region, Russia; U-W: *Rotulispira damesii* (Noetling, 1885), clockwise coiling. PIN 5204/13, Cenomanian (100-94 Ma), Orel region, Russia: U – view from upper side, V – view from lower (attachment) side, W – lateral view; X-Z: *Tectorotularia* cf. *polygonalis* (J. de C. Sowerby, 1829), PIN 5204/6, Aptian (125-113 Ma), Atyrau region, Kazakhstan: X – view from upper side, Y – view from the attachment side, Z – lateral view. Material is deposited in the Paleontological Institute of Russian Academy of Sciences (PIN) and the Natural History Museum, Geological Museum, University of Tartu, Estonia (TUG). Scale: A-C – 10 mm, D-K – 5 mm, L, M – 10 mm, N-Z – 5 mm.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Late Triassic epoch. The Late Triassic (Carnian) genus *Corynotrypoides*, characterized by tiny quickly branching tubes forming procumbent pseudocolonies and originally described as bryozoan (see Taylor, 2014), seems too be reasonably close to *Filograna*. At least some of the Triassic serpulids were members of reef communities, and some of them were even reef-forming organisms (e.g. Braga and Lopez-Lopez, 1989).

In total, only about 10 species are known from the Late Triassic (e.g. Ziegler and Michalík, 1980; Senowbari-Daryan and Link, 2005; Senowbari-Daryan et al., 2007), but due to the relatively small size of tubes, Triassic fossil diversity is poorly studied. Morphological diversity of this period includes several characteristic types similar to Recent forms, suggesting that at least some extant genera have their evolutionary roots in the Triassic. The presence of *Filograna*-like fossils indicates that not only clade B was already separated from clade A by this time, but inside clade BI the *Protis-Filograna* clade had already diverged from the *Chitinopoma-Protula-Metavermilia-Vermiliopsis* clade by the end of the Triassic (fig. 9). Probable members of the latter group are small triangular to pentangular tubes described as "*Serpula* spec. indet." by Jaworski (1915). Interestingly, in the earliest known *Filograna* (*F. minor* Senowbary-Daryan et al., 2007) from the Middle Triassic, tubes of individual specimens are not yet densely connected to each other, while in Late Triassic species the integration of individuals is more prominent (see Senowbari-Daryan and Link, 2005). This may mean that early evolution of the *Filograna*/*Salmacina* clade and its divergence from other serpulids occurred shortly before the Middle Triassic.

Figure 8. Morphological diversity of Mesozoic (Late Cretaceous) and earliest Cenozoic tube-dwelling polychaetes. A, B: calcareous sabellid *Glomerula serpentina* (Goldfuss, 1831): A – cross-section, showing trilobate lumen, GPI HH 4402, latest Maastrichtian (~66 Ma), Maastricht region, Netherlands (after Jäger, 2005: Pl. 1, fig. 6); B – specimen with characteristic meandrous coiling, GPI HH 2556, Early Maastrichtian (~71 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 2, fig. 2). C-F: possible members of clade BI: C, D – "*Filogranula*" *cincta* (Goldfuss, 1831): C – BGR/NLfB kma 324, Late Maastrichtian (~70 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 8, fig. 10); D – SCMH 782, Coniacian (~88 Ma), Helgoland Island, Schleswig-Holstein, Germany (after Jäger, 1991: Pl. 5, fig. 1a). E – *Metavermilia* (*Vepreculina*) *minor* Jäger, 1983, holotype, BGR/NLfB kca 46, Early Campanian (~80 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 9, fig. 8b). F – *Vermiliopsis fluctuata* (J. de C. Sowerby, 1829), BGR/NLfB kma 321, Early Maastrichtian (~70 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 8, fig. 2a). G-U – possible members of AII clade: G, H – *Dorsoserpula wegneri* (Jäger, 1983); G – aperture with "Nebenröhre", additional tube of uncertain nature, GPI GÖ 843-4, Campanian or Early Maastrichtian (~83-72 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 4, fig. 5); H – holotype, characteristic coiling mode around crinoid stem object, BGR/NLfB ksa 15, Late Santonian (~84 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 4, fig. 1a); I – *Neovermilia ampullacea* (J. de C. Sowerby, 1829), SCMH 885, Turonian or Coniacian (~94-86 Ma), Helgoland Island, Schleswig-Holstein, Germany (after Jäger, 1991: Pl. 1, fig. 4c); J – *Parsimonia parsimonia* Regenhardt, 1961, spirally coiled modification, GPI GÖ 843-3, Middle Santonian (~85 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 3, fig. 4a); K, L – *Pyrgopolon* (*Septenaria*) *macropus* (J. de C. Sowerby, 1829), GPI HH 2577, Early Maastrichtian (~71 Ma), Rügen Island, Mecklenburg-Western Pomerania, Germany (after Jäger, 1983: Pl. 10, fig. 8b,d); M, N – *Pyrgopolon* (*Hamulus*) *sexangularis* (Münster in Goldfuss, 1831), GPI GÖ 843-8, Late Campanian (~74 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 11, fig. 11d, a); O, P – *Pyrgopolon* (*Pyrgopolon*) *mosae mosae* de Montfort, 1808; O – GPI HH 4427, latest Maastrichtian (~66 Ma), Maastricht region, Netherlands (after Jäger, 2005: Pl. 7, fig. 3); P – base of broken tube showing tubulae, NHMM 2001 101, Late Maastrichtian (~67 Ma), Maastricht region, Netherlands (after Jäger, 2005: Pl. 7, fig. 1); Q-R – operculum of *Pyrgopolon* (*Pyrgopolon*) *mosae ciplyana* (de Ryckholt, 1852), from private collection, Late Maastrichtian (~68 Ma), Maastricht region, Netherlands (after Jäger, 2005: Pl. 7, fig. 7b,a); S – *Pyrgopolon* (*Pyrgopolon*) *regia regia* Regenhardt, 1961, NHMM JJ 882b, Late Maastrichtian (~68 Ma), Belgium (after Jäger, 2005: Pl. 8, fig. 6b); T – *Pyrgopolon* (*Septenaria*) *polyforata* (Jäger, 1983, BGR/NLfB kma 335, Early Maastrichtian (~70 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 10, fig. 11); U – *Ditrupa schlotheimi* (Rosenkrantz, 1920), NHMM 1992200-2, Early Danian (~66-65 Ma), Belgium (after Jäger, 1993: Pl. 4, fig. 2). V-W: questionable members of clade AII: V – *Pentaditrupa subtorquata* (Münster in Goldfuss, 1831), BGR/NLfB kma 309, Early Maastrichtian (~71 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 7, fig. 2); W – *Nogrobs* (*Tetraditrupa*) *canteriata* (von Hagenow, 1840), GPI BN 2 GPI Bo M. Jäger, Early Maastrichtian (~71 Ma), Rügen Island, Mecklenburg-Western Pomerania, Germany (after Jäger, 1983: Pl. 7, fig. 10). X-HI: clade uncertain, taxa with obligatory spiral coiling: X-Y – *Conorca trochiformis* (von Hagenow, 1840), GPI HH 2588, Early Maastrichtian (~72 Ma), Schleswig-Holstein, Germany (after Jäger, 1983: Pl. 13, fig. 8a, b); Z – *Cycloplacostegus pusillus* (J. de C. Sowerby, 1844), GPI HH 2582, latest Campanian (~73 Ma), Schleswig-Holstein, Germany (after Jäger, 1983: Pl. 12, fig. 11); AB-BC – *Protectoconorca senonensis* Jäger, 1983, holotype, GPI HH 2609, Middle Santonian (85 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 16, fig. 2a,b); CD – *Rotularia tobar gracilis* Jäger, 1993, holotype, NHMM 1992201-1, Early Danian (~66-65 Ma), Belgium (after Jäger, 1993: Pl. 5, fig. 1); DE – *Orthoconorca turricula* (d'Eichwald, 1865), GPI HH 2593, Early Maastrichtian (~72 Ma), Schleswig-Holstein, Germany (after Jäger, 1983: Pl. 14, fig. 3); EF – *Neomicrorbis crenatostriatus subrugosus* (Münster in Goldfuss, 1831), lectotype, GPI BN 5 GPI Bo M. Jäger; Late Campanian (~73 Ma), North Rhine-Westphalia, Germany (after Jäger, 1983: Pl. 15, fig. 9a); FG-HI: *Neomicrorbis crenatostriatus crenatostriatus* (Münster in Goldfuss, 1831): FG - BGR/NLfB (G), Nr. kma 351, Early Maastrichtian (~71 Ma), Lower Saxony, Germany (after Jäger, 1983: Pl. 15, fig. 2a); GH-HI – operculum, GPI HH 2604, Early Campanian (~83 Ma), Schleswig-Holstein, Germany (after Jäger, 1983: Pl. 15, fig. 6b,a). IJ-KL: genuine spirorbins: IJ – *Bipygmaeus pygmaeus* (von Hagenow, 1840), GPI HH 4434, latest Maastrichtian (~66 Ma), Maastricht region, Netherlands (after Jäger, 2005: Pl. 8, fig. 13a); JK-KL – *Neodexiospira palaeoforaminosa* Jäger, 2005, latest Maastrichtian (~66 Ma), Maastricht region, Netherlands: JK – GPI HH 4437 (after Jäger, 2005: Pl. 8, fig. 17); KL – GPI HH 4438 (after Jäger, 2005: Pl. 8, fig. 18b). Material is deposited in the Geologisch-Paläontologisches Institut und Museum der Universität Hamburg (GPI HH), Geozentrum Hannover (formerly: Bundesanstalt für Geowissenschaften und Rohstoffe/Niedersächsisches Landesamt für Bodenforschung, Hannover) (BGR/NLfB), Geowissenschaftliches Zentrum der Universität Göttingen (formerly: Geologisch-Paläontologisches Universitäts-Institut, Göttingen) (GPI GÖ); Natuurhistorisch Museum Maastricht (NHMM); Steinmann-Institut für Geologie, Mineralogie und Paläontologie der Universität Bonn (formerly: Geologisch-Paläontologisches Universitäts-Institut), Bonn (GPI BN); Stühmer collection in the Museum Helgoland (SCMH). Scale: A – 0.5 mm, B-H, K-S, U, V, X-Z, CD-KL – 1 mm, I, J, T, W, AB, BC – 5 mm.


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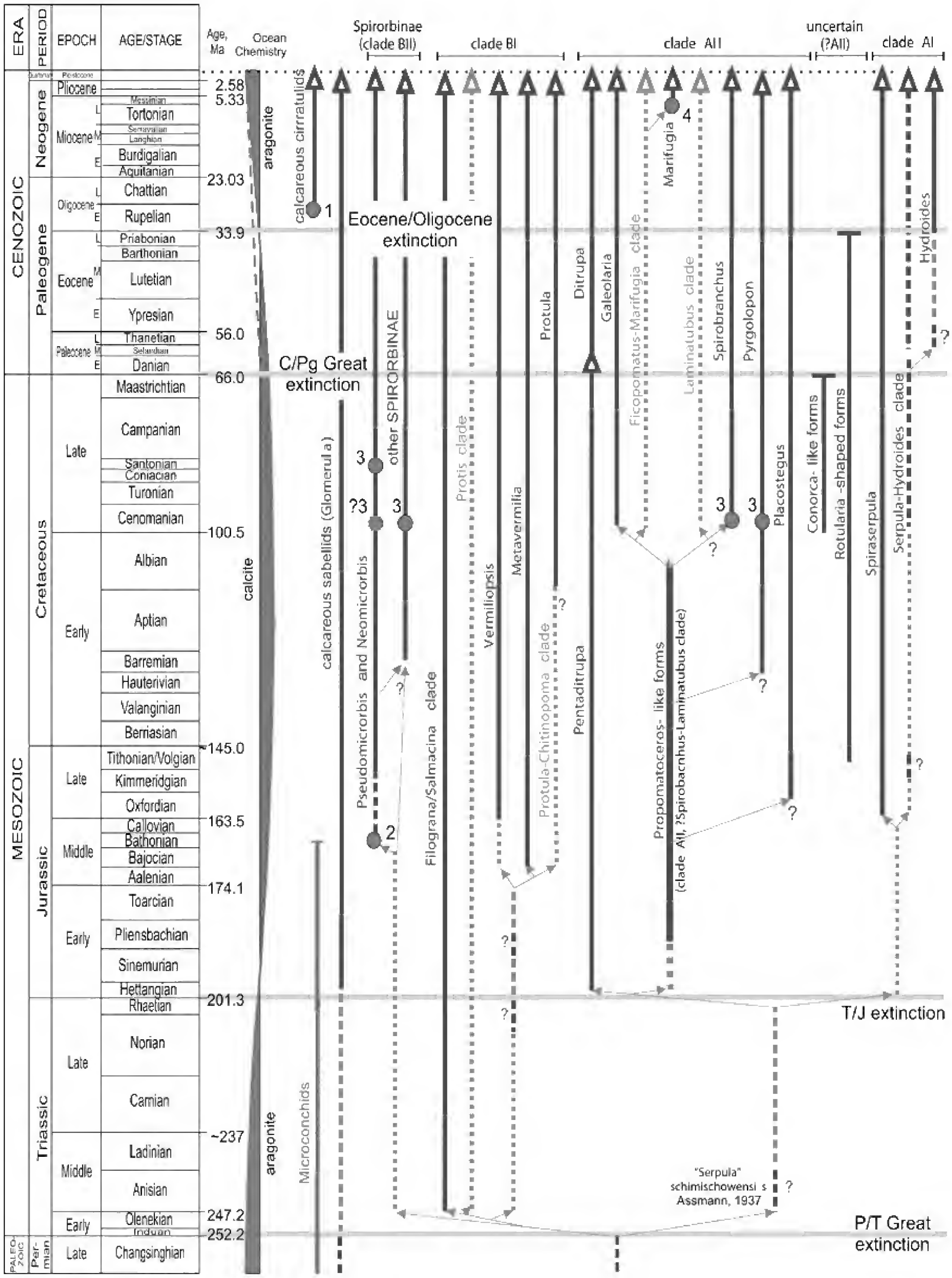

Figure 9. Geological history of calcareous tube-building polychaetes in Mesozoic and Cenozoic suggested by fossil record. Only the most common serpulid genera and those from the phylogenetic tree (fig. 1) are included. For legend see Figure 6. Major events: 1 – most ancient finds of cirratulids with calcified tubes; 2 – the youngest possible position of "coiling point" in spirorbins; 3 – first finds of calcified opercula in several serpulid lineages; 4 – penetration of serpulids to freshwater cave habitat.

Whether triangular tubes with single keels on the upper side (e.g. "*Propomatoceros*" *slavicus* Ziegler and Michalík, 1980) and large-sized tubes with round cross-section ("*Serpula*" *constrictor* Winkler, 1861 *sensu* Jaworski, 1915) from Indonesia are representatives of clade A or clade BI is uncertain. "*Serpula*" *schimischowensis* Assmann, 1937, characterised by large tubes with one or two indistinct keels, is probably the only Triassic species that can be confidently interpreted as a member of clade A (either AI or AII). However, most Triassic finds cannot be attributed to any particular clade.

In conclusion, serpulids did not seem to play a significant role in Middle Triassic ecosystems, and their wide diversification and world-wide dispersal began during the Late Triassic to the Early Jurassic (237-174 Ma). Calcareous tubes first appeared in sabellids and serpulids either in the Late Paleozoic or during the Triassic as an adaptation to predation pressure and evolved in rapidly changing post-Permian/Triassic extinction ecosystems. The main evolutionary trends suggested by Triassic finds are size diversification that resulted in appearance of large tubes, including irregularly coiled attached ones, and wide dispersal of pseudocolonial forms. However, all known Triassic serpulid localities are restricted to the margins of the warm Tethys Ocean that extended sub-latitudinally from South Europe to Indonesia.

3.5. Jurassic (201-145 Ma) diversification epoch

Serpulid faunas of the Jurassic are relatively well studied. Taxonomical reviews describing morphological variety of fossil tubes are mostly based on European material (Parsch, 1956; Ippolitov, 2007a, b; Jäger and Schubert, 2008) with most species known since the 19th century (e.g. Goldfuss, 1831).

The Triassic/Jurassic boundary is characterised by the large extinction event, but its influence on serpulid biota has not been studied. In the Early Jurassic (201-174 Ma) new serpulid morphotypes include larger sculptured subtriangular to sub-pentangular attached tubes with prominent median keels (genus *Propomatoceros*) and free-lying pentagonal tubes (genus *Pentaditrupa*; see Jäger, 2005; Jäger and Schubert, 2008). During the Early Jurassic epoch, serpulids, including *Filograna*-like forms (Aberhan, 1992) seem to disperse from Europe to South America (Behrendsen, 1891; Biese, 1961). The most ancient finds of free-lying tetragonal serpulids of the genus *Nogrobs* are known from South America (Behrendsen, 1891) and dated by Late Pliensbachian (~185 Ma), while finds of this genus in Europe are somewhat younger (Late Toarcian; ~176 Ma; Jäger, 2005). During the Early Jurassic, serpulids also first dispersed to temperate waters of Northern Hemisphere, appearing in North Siberia (Ippolitov, unpubl.; Kirina, 1976: 98). In Canada diversified Boreal serpulid communities are known starting from the Middle Jurassic (Bathonian/Callovian boundary, ~166 Ma; Parsch, 1961).

During the Middle-Late Jurassic (174-145 Ma) the total number of known serpulids increased up to about 150 nominal species (Parsch, 1956; Ippolitov, 2007a, b; 2010), but the exact number is uncertain because many taxa are in need of revision. This was the time of remarkable radiation in Mesozoic (Ippolitov, 2010), which included the appearance of most important serpulid morphotypes, such as forms with multiple keels and spiral tubes (Ippolitov, 2010; also fig. 7). The earliest representatives of many extant genera (e.g. *Vermiliopsis*, *Nogrobs*, *Metavermilia*, *Spiraserpula*) can be recognised with confidence in the Jurassic (Jäger, 1983; 1993; 2005; Ippolitov, 2007a, b; 2010; Vinn and Wilson, 2010).

Comparison of the fossil record of this age with the molecular phylogeny of Recent taxa (fig. 1) shows that Middle Jurassic fossil faunas already contain members of all three major clades, and even smaller clades, including some extant genera, can be recognised (fig. 9). Clade BI is represented by numerous small to medium-sized tubes with several keels classified as *Vermiliopsis* and *Metavermilia*. The first members of these genera are confidently traced from the Middle Jurassic (*Metavermilia goldfussi* Ippolitov, 2007a and *Vermiliopsis negevensis* Vinn et Wilson, 2010) starting from the Bajocian (~170 Ma). There are earlier records of *Metavermilia*-like tubes from the Late Triassic (Rhaetian; 208.5-201 Ma) and Pliensbachian (191-183 Ma) (Jäger, 2005: 148), but because these finds remain undescribed, they are considered here as members of *Metavermilia-Vermiliopsis* clade (fig. 9) or its stem group. During the Late Jurassic the morphogroup *Metavermilia-Vermiliopsis* (fig. 7H-J) was represented by numerous species (Goldfuss, 1831; Parsch, 1956), suggesting that all main divergence events in the *Chitinopoma-Protula-Metavermilia-Vermiliopsis* clade happened before the end of the Jurassic. However, unequivocal members of the *Protula-Chitinopoma* clade were still not present in the Jurassic, probably indicating that divergence within this branch took place later. Another member of clade BI, *Filograna*/*Salmacina* (fig. 7F, G) was common in the Jurassic continuing from the Triassic. Non-attached and variously curved tubes were widely spread in the Early Jurassic (Jäger, 1993). Some of them, e.g. "*Serpula*" *etalensis* (Piette, 1856), have tubes with round cross-sections and numerous peristomes, thus resembling free anterior parts of Recent deep-sea *Bathyvermilia* (ten Hove, pers. comm. 2014) belonging to clade BI. The affinity of "*Serpula*" *etalensis* with this clade is supported by simple unilayered wall with irregularly oriented prismatic (IOP) (Vinn et al., 2008c) structure, which is characteristic for members of clade BI.

Clade AI is represented in the Jurassic by *Spiraserpula*. The most ancient probable member of this genus is *Spiraserpula oligospiralis* Ippolitov, 2007b (fig. 7P) from the Middle-Upper Jurassic boundary (Late Callovian/Early Oxfordian; 163.5 Ma), which has characteristic tube coiling, but no ITS typical for younger (Cretaceous to Recent) members of the genus. There are numerous doubtful records of this genus and related *Cementula* from the Early-Middle Jurassic (see Jäger, 1993; Ippolitov, 2007b; Jäger and Schubert, 2008) and even Triassic (Ziegler and Michalik, 1980). Because all these pre-Callovian tubes do not have typical subtriangular cross-sections with median keel extending into a spine over the aperture, these records may belong either to the representatives of the calcareous sabellid *Glomerula* (that tends to have spirally coiled tubes as juveniles) or to a yet undescribed genus. The presence of well-defined *Spiraserpula* in Middle-Late Jurassic indicates that true representatives of the *Serpula-Hydroides* clade must have already existed at that time, but most fossil species can hardly be placed within these

genera. The probable exception is Late Jurassic (Tithonian, ~150 Ma) *Serpula coacervata* Blumenbach, 1803, which is similar in morphology to some Recent *Serpula* species and also produced tube aggregations (ten Hove and van den Hurk, 1993). Another possible clade AI member of Late Oxfordian age (~158 Ma), belonging to still undescribed species, can be seen in fig. 7Q.

Clade AII is represented by the well-recognizable genus *Placostegus* traced from the Late Oxfordian (~158 Ma: *Placostegus conchophylus* Radwańska, 2004). Like Recent forms, fossil *Placostegus* spp. already had transparent tubes (Ippolitov, unpubl.) Other transparent tubes of the same age are usually classified as *Filogranula* (fig. 7K) (see Ippolitov, 2007a) and are known from the latest Early Jurassic and Middle Jurassic ("*Serpula tricristata*" Goldfuss, 1831: Early Toarian to earliest Aalenian, ~180-174 Ma). Given that tube transparency is produced by simple prismatic (SP) structure (Vinn et al., 2008b) and that all non-spirorbin Recent species having this structure are members of clade AII (Vinn and Kupriyanova, 2011), fossil transparent tubes can be interpreted as belonging to members of clade AII, probably related to the *Placostegus* and *Vitreotubus*. Data on tube ultrastructures of some fossil species with quadrangular tubes (Vinn and Furrer, 2008; Vinn et al., 2012) show that such tubes also have SP structure, thus confirming attribution of such tubes to clade AII. Another possible member of the clade AII is *Neovermilia* (fig. 7O) that, like *Placostegus*, is known from the Late Oxfordian (Radwańska, 2004).

The *Ditrupa-Pseudochitinopoma* group is another subclade within clade AII with possible roots in the Jurassic period. Small tubes with characteristic more or less regular transverse ridges and circular cross-section, closely resembling Recent *Pseudochitinopoma beneliahuae* Kupriyanova et al., 2012, are known from the Late Callovian or Early Oxfordian (~164-163 Ma; Ippolitov, unpubl.) of Crimea. Although representatives of true *Ditrupa* appear only after the Cretaceous-Paleogene boundary (Jäger, 1993 and fig. 8U), from the beginning of the Early Jurassic (Hettangian; ~200 Ma) there are records of *Pentaditrupa* (Jäger and Schubert, 2008), a genus with free-lying pentagonal tubes considered as a likely direct ancestor of *Ditrupa* (see Jäger, 1993: 92; Jäger and Schubert, 2008: 56).

Numerous fossils having large sub-triangular tubes with pronounced median keels appear during the Early Jurassic. They are classified within the exclusively "fossil" genus *Propomatoceros* (fig. 7L, M) and related *Mucroserpula* (Ippolitov, 2007b; Jäger and Schubert, 2008). Tube ultrastructures of *Propomatoceros* show a dense outer layer (*sensu* Vinn and Kupriyanova, 2011) formed by spherulitic prismatic structure (SPHP; fig. 5G), typical for clade A. Despite the striking morphological similarity of these tubes to Recent *Spirobranchus*, fossil *Propomatoceros* seem to lack opercular calcification, therefore, its attribution to any of Recent genera is not justified (Ippolitov, 2007b). Jurassic *Propomatoceros* appears to be a member of *Laminatubus-Spirobranchus* clade (fig. 1) or a stem group including common ancestors of *Laminatubus-Spirobranchus* and *Galeolaria-Ficopomatus-Marifugia* clades.

In addition to the morphotypes well-represented in Recent biota, large spirally coiled tubes adapted for settlement on small objects with subsequent transition to free-lying on soft substrates originated during the Jurassic (Jäger, 1993). Such tubes became an essential component of serpulid faunas in late Mesozoic (Cretaceous) seas. It seems that during the Jurassic such a morphotype has appeared at least twice: in the Early Jurassic (*Nogrobs s. str.* with tetragonal tubes) and in the Late Jurassic (Kimmeridgian; ~155 Ma) of Austral Realm (*Austrorotularia* with three-keeled tubes). The phylogenetic position of these genera is uncertain. Fossil *Nogrobs* seems to be a member of clade AII according to its transparent tube with simple prismatic (SP) structure (Kupriyanova and Ippolitov, 2012). However, Recent members of the genus, *Nogrobs grimaldii* (Fauvel, 1909), have opaque tubes (*ibid.*), which makes matching of Recent and fossil forms doubtful. Tubes of *Austrorotularia* by their size and type of sculpture are comparable with those of Recent *Spirobranchus*, thus, *Austrorotularia* is likely to belong to clade AII as well. Although Jäger (1993: 86-87) suggested an evolutionary transition from *Nogrobs* to *Austrorotularia* and other genera formerly included in *Rotularia* as subgenera (see Regenhardt, 1961; Jäger, 1993), the tube sculptures in all these taxa are too different, suggesting that coiling in all these taxa could have evolved independently within clade A. Comparative ultrastructural study of all former *Rotularia* subgenera is still pending, but at least one genus, *Rotularia sensu stricto* from the Paleogene, shows distinct advanced lamello-fibrillar (LF) structure in the tube wall (Vinn, 2008), which is quite difficult to connect with simple prismatic structure of *Nogrobs*.

To conclude, although Jurassic was the epoch of rapid diversification of serpulids and their world-wide dispersal, subtropical latitudinal Tethys Ocean remained the main centre of dispersal thoughout the entire Jurassic.

3.6. Cretaceous (145-66 Ma): further diversification

During the Cretaceous period (145-66 Ma) the number of nominal species increased to over 200 (e.g. Jäger, 1983; 1993; 2005; Ippolitov, 2010). The Cretaceous serpulid fauna is relatively well-studied (Brünnich Nielsen, 1931; Regenhardt, 1961; Chiplonkar and Tapaswi, 1973a, b; Lommerzheim, 1979; Jäger, 1983; 1993; 2005; Ziegler, 1984; Kočí, 2009; 2012 and many more papers) and was subject to elaborate classification of fossil tubes under Recent generic names. However, the serpulid fossil record of the Early Cretaceous epoch (145-100 Ma) is still very fragmentary, with large unstudied gaps, while the Late Cretaceous epoch (100-66 Ma) is probably the best-studied time interval in serpulid evolutionary history, characterised by a very continuous fossil record.

Excluding scarce data scattered over older publications (e.g. Regenhardt, 1961, who redescribed, amongst others, some Early Cretaceous serpulids and introduced several new taxa), there are only three comprehensive investigations analysing serpulid faunas of the Early Cretaceous. The generic composition of the serpulid community from the Hauterivian (~132 Ma) of South America (Garberoglio and Lazo, 2011; Luci et al., 2013) looks basically similar to that of the Jurassic. The only innovation is the abundance of coiled *Neomicrorbis*/

Pseudomicrorbis that were extremely rare in the Jurassic. The fauna of Barremian age (~128 Ma) described by Jäger (2011) from South-Eastern France differs from Late Jurassic serpulid biota and resembles that of the Late Cretaceous. Besides *Neomicrorbis* (fig. 8EF-HI) and its possible ancestor *Pseudomicrorbis* (fig. 7R), it includes diversified spirorbins as well as large tubes of *Pyrgopolon* (fig. 8K-T) and characteristic small *Vepreculina* (treated as subgenus of *Metavermilia* by Jäger, 1993; 2005; 2011; see fig. 8E), both unknown in the Jurassic. The younger Early Aptian (~125-120 Ma) fauna from England (Ware, 1975), however, again resembles the Jurassic one, as no genera such as *Neomicrorbis* and *Pyrgopolon* were present. This is probably because the territory of England was part of the cold-water Boreal realm, while the major serpulid diversification took place in the warmer Tethyan Realm. Also, because this community inhabited sponges as a substrate, direct comparisons with communities found on other substrates are not really confident. The early Cretaceous was also the time of wide divergence of *Rotularia*-like coiled serpulids, represented now by *Austrorotularia, Tubulostium* (both in Southern Hemisphere only), *Rotulispira* and *Tectorotularia.*

The Late Cretaceous was the time when warm epicontinental seas characterised by high rates of carbonate sedimentation occupied large areas in Europe. Serpulid evolution of this time has been described in detail by Jäger (2005: 210-212). The main changes in the serpulid biota include diversification of species within older genera and shifts of dominant genera. Because of the carbonaceous mud floor of Late Cretaceous European seas, this time period was dominated by forms quickly starting to grow upwards, such as the large *Pyrgopolon*, and free-lying forms like *Pentaditrupa* (fig. 8V) and *Nogrobs* (*Tetraditrupa*) (fig. 8W) that did not need much space to attach their initial tubes. Some *Pyrgopolon* species, such as hexagonal members of the subgenus *Hamulus* (fig. 8M-N), adapted to a new lifestyle by modifying their tube sculpture into a peculiar "snow shoe" shape *sensu* Savazzi (1995), which allowed animals to live free on the surface of a muddy substrate (see discussion of "*Serpula*" *alata* in Savazzi (1995; 1999)). The deficit of hard substrates probably also explains appearance of numerous genera with spiral tubes that cannot be attributed to any Recent genus (e.g. *Conorca, Orthoconorca,* and *Protectoconorca,* see fig. 8X, Y, AB, BC, DE) as well as diversification of *Placostegus*-like taxa normally growing upwards from the substrate (fig. 8Z). On the contrary, large spiral *Rotularia*-shaped forms, the common element of serpulid biota during the Early Cretaceous and earliest Late Cretaceous (Cenomanian; 100-94 Ma), almost disappeared in European communities starting from the base of Turonian (~94 Ma), probably being displaced by *Conorca*-like forms (Jäger, 1993). However, in epicontinental seas of former Gondwana continent in the Southern Hemisphere during the Mesozoic, coiled free-lying forms remained the dominant morphotype during the entire Late Cretaceous epoch (e.g. see Tapaswi, 1988 for India and Macellari, 1983 for Antarctica).

Large tubes having pronounced median keels (clade AII) and mostly attached to the substrate (*Propomatoceros*-like forms) became less common in the Cretaceous than they were in the Jurassic. Finds of *Spirobranchus*-like opercula (Lommerzheim, 1979) starting from the earliest Late Cretaceous (Cenomanian; 100 Ma) indicate that this clade probably diverged from the *Laminatubus* lineage before that time. However, because *Spirobranchus* is hardly distinguishable from Jurassic *Propomatoceros* by tube morphology, further studies are needed to date this transition.

Starting from the end of Early Cretaceous (Early Albian; ~110 Ma; Jäger, 2005), records of large unsculptured *Protula*-like tubes (clade BI) become common. However the origin of this genus should be hypothesised cautiously because simple unsculptured tubes of *Protula* are hardly recognisable among fossils of Early Cretaceous and Jurassic. *Protula*-like tubes are common in the Albian and Cenomanian (100-94 Ma), but almost completely disappear in shallow-water European seas starting from Turonian and up to the end of Late Cretaceous (94-66 Ma). The first representatives of another BI member, characteristic tiny-sized serpulid genus *Josephella*, are known from the Late Cretaceous of Europe (Regenhardt, 1961; Jäger, 2005).

During the Cretaceous, opercular calcification appeared in several independent lineages (*Neomicrorbis* and other Spirorbinae (fig. 8GH-HI); *Spirobranchus-Galeolaria* clade and *Pyrgopolon* (fig. 8Q, R)) (Wade, 1922; Avnimelech, 1941; Lommerzheim, 1979; Cupedo, 1980a, b; Jäger, 1983; 2005), supposedly improving protection against predators.

3.7. The rise of Spirorbinae

The earliest spirorbins, represented by characteristic large-sized *Neomicrorbis* tubes (up to 6-7 mm in diameter) bearing numerous longitudinal rows of tiny tubercules appear to be of Early Cretaceous age (?Early Hauterivian, ~132 Ma, Luci et al., 2013; Late Barremian, ~126 Ma, Jäger, 2011; Late Berriassian, ~141 Ma, Ippolitov, unpubl.). Undescribed finds mentioned by Jäger (2005) from the Middle Jurassic (Late Bathonian; ~166 Ma) also seem to belong to *Neomicrorbis* (Jäger, unpubl.). It is unclear whether the Late Jurassic (?Middle Kimmeridgian, ~154 Ma) "*Spirorbis clathratus*" Étallon, 1862 *sensu* von Alth, 1882 belongs to *Neomicrorbis* or to the closely related *Pseudomicrorbis* (fig. 7R). The latter genus is similar to *Neomicrorbis,* but its tube sculpture is represented by rows of very small pits, not tubercules, and the initial tube is straight. For the latter character *Pseudomicrorbis* was originally placed outside Spirorbinae (Jäger, 2011), however, in Recent Spirorbinae the initial tube is also straight or just slightly curved (Rzhavsky, pers. comm., 2013; Malaquin, 1904: fig. 1; Okuda, 1946: Pl. 26, fig. 16; ten Hove, 1994: 66). Whether *Pseudomicrorbis* belongs within or outside Spirorbinae depends on a formal definition of spirorbins, but *Pseudomicrorbis* is clearly closely related to *Neomicrorbis.* The only known Recent species of this group, *Neomicrorbis azoricus*, combines characters typical for spirorbins and non-spirorbin serpulids, so its attribution to spirorbins is uncertain (ten Hove and Kupriyanova, 2009: 66; Rzhavsky, pers. comm.).

Abundant undisputable spirorbins similar to extant forms appear from the middle of the Early Cretaceous (Late Barremian, ~126 Ma, Jäger, 2011). These finds are represented by anticlockwise coiled sculptured species questionably referred to *Neodexiospira* (mentioned as "*Janua* (*Dexiospira*)?"), and clockwise coiled unsculptured tubes described as *Pileolaria*? spp.

From the latest Cretaceous (~66 Ma) spirorbins, again attributed to *Pileolaria*? and *Neodexiospira* (fig. 8JK-KL), the latter with good confidence due to characteristic sculpture and preserved opercula associated with tubes (Jäger, 2005), together with exclusively fossil genus *Bipygmaeus* (fig. 8IJ), became common among encrusters (e.g. Jäger, 1983; 1993; 2005). Younger Early Paleogene (62-59 Ma) communities (Lommerzheim, 1981) already contain diversified spirorbins.

The intensive radiation of Spirorbinae can be attributed to their small size, short generation time, and compact spiral tubes allowing them to quickly colonise flexible and ephemeral substrates, such as macroalgae and seagrass blades, and thus, to compete for settlement sites in the highly productive and densely populated upper subtidal zone (Ippolitov, 2010). Spirorbinae were not the only Mesozoic serpulids adapted to settlement on algae, also some larger forms twisted over algal blades. Other Mesozoic serpulids that experimented with coiling were *Rotularia*-shaped forms (*Austrorotularia, Rotulispira, Tectorotularia, Tubulostium*) and *Nogrobs sensu stricto* with large planospiral tubes adapted to soft sediments, as well as small *Conorca*-like tubes (*Conorca*, *Orthoconorca*, *Protectoconorca*) often coiled in high turret-like spirals. The latter forms probably disappeared due to being outcompeted by Spirorbinae.

The origin of Spirorbinae is still a challenge for paleontologists because fossil data do not agree with molecular phylogenies. As pseudocolonial serpulids representing the *Filograna/Salmacina* clade are common in the Middle and Late Triassic, the spirorbin lineage that apparently diverged early within "filogranin" clade BI (fig. 1) should have appeared even earlier, far from the Late Jurassic to Early Cretaceous age postulated by paleontological data. But the divergence point does not necessarily coincide with the "coiling point", which possibly occurred later in this lineage (fig. 9).

3.8. Cenozoic (66 Ma) to Recent: the rise of Recent serpulid fauna

The serpulids seem to cross the Cretaceous-Paleogene boundary (66 Ma) without any drastic losses, even though this boundary is famous for its extinction event killing numerous other marine groups and the dinosaurs. A detailed study of the Maastrichtian-Danian boundary interval (around 66 Ma) by Jäger (1993) has shown no drastic changes in serpulid faunas around the boundary. However, reshaping of post-crisis marine ecosystems of the early Cenozoic might have indirectly triggered further radiation of serpulid biota. At least some genera seem to completely disappear during the latest Cretaceous (Table 2; see also Jäger, 1993), but whether this is a true extinction pattern or an artifact of our poor knowledge of the Early Cenozoic serpulid faunas, remains unclear.

The number of serpulid species increased in the Paleogene (66-23 Ma), but the fauna of this period is relatively poorly studied. Paleogene serpulid diversity was studied by Brünnich Nielsen (1931), who described a fauna of mostly attached serpulids from the Danian (mostly Middle Danian; ~64-63 Ma) of Denmark. His data show that faunas of Paleogene are comparable to those of Late Cretaceous age, as many genera and dominating morphotypes (*Neomicrorbis, Pyrgopolon, Spirobranchus*-like forms, *Protula*) remain common.

Starting from Danian there was a remarkable come-back of coiled forms (represented now by *Rotularia sensu stricto*), which continued throughout the entire Paleocene and Eocene (66-34 Ma; Jäger, 1993; Wrigley, 1951). At least in some fossil communities of the Middle Paleocene (62-59 Ma), spirorbin diversity is similar to that of non-spirorbin serpulids, indicating their intensive diversification (e.g. Lommerzheim, 1981).

The influential, but clearly outdated monograph on serpulid faunas of the Cenozoic including Eocene (56-34 Ma) and Oligocene (34-23 Ma) epochs by Rovereto (1904) treats materials from Western Europe and Italy. In general, serpulid fauna of this age resembles that described by Brünnich Nielsen (1931) from the Paleocene. Rovereto (1904: Pl.3, fig. 3) figures at least one remarkable loop-coiled species of Eocene age (56-34 Ma) that closely resembles Recent *Hydroides*, the genus not known from older Mesozoic sediments. Gradual expansion of free-lying *Ditrupa* in Europe started from the earliest Paleogene and peaked in the Eocene (~56-34 Ma). Also, during the Eocene *Pyrgopolon* tubes that can be traced back to the Cretaceous, but are remarkably smaller, became common and diverse at least in some regions (Wrigley, 1951).

The Eocene/Oligocene boundary, the largest extinction event in the Cenozoic, was also an important time in serpulid evolution (Jäger, 2005: 211). Some taxa that once flourished in Mesozoic seas have gradually lost their dominance in the calcareous tubeworm communities by this time. The most remarkable example is the calcified sabellid *Glomerula*, traced up to the end of Eocene (34 Ma) and nowadays known as a single species endemic to the Great Barrier Reef. Other examples include free-lying coiled *Rotularia*, which completely disappeared by the end of Eocene (34 Ma; Jäger, 1993: 88) and problematic *Neomicrorbis*, still present in Recent seas as a single bathyal relict species (Zibrowius, 1972; ten Hove and Kupriyanova, 2009).

To summarise, during the entire Paleogene period there were no drastic evolutionary experiments with tube shape and coiling comparing with the Mesozoic, but there were obvious shifts in dominance of serpulid communities. However, the most ancient calcareous tubes of cirratulids are known from the late Oligocene (~25 Ma) in North America (Fischer et al., 1989; 2000), suggesting that cirratulids acquired tube calcification quite late and independently from serpulids and sabellids (Vinn and Mutvei, 2009).

Serpulid communities of the younger Cenozoic (Neogene period; 23-2.6 Ma) are very similar to those found in Recent seas. Several hundreds of fossil serpulid species have been described from the Neogene (e.g. Rovereto, 1899; 1904; Schmidt, 1950; 1951; 1955; Radwańska, 1994a). The important new element compared to Mesozoic faunas is the wide dispersal of the *Hydroides* morphotype (slowly growing tubes with flattened upper side and loop-coiling tendency). *Hydroides* probably had appeared during the early Paleogene (e.g. Lommerzheim, 1981) or Eocene (Rovereto, 1904) and became common starting from the Neogene (Rovereto, 1899; 1904; Schmidt, 1950; 1951; 1955; Radwańska, 1994a).

During the latest Cenozoic serpulids colonised freshwater cave habitats. The most ancient fossilised tubes of the only known Recent freshwater species *Marifugia cavatica* Absolon

and Hrabě, 1930 were discovered in a collapsed cave in Slovenia are dated around the Late Pliocene/earliest Pleistocene (2.5-3.6 Ma; Bosák et al., 2004). Molecular data of Kupriyanova et al. (2009) suggest that penetration into non-marine waters appeared once in the evolution of Serpulidae. The transition of *Marifugia* to a subterranean environment likely has occurred via ancestral marine shallow-water to intertidal or estuarine species (like extant *Ficopomatus*) that evolved the necessary adaptations to withstand low salinity and then penetrated freshwater caves via surface lakes (Kupriyanova et al., 2009). The age of serpulid penetration of brackish water is uncertain as there is no reliable fossil record of the brackish-water genus *Ficopomatus*. Two Cenozoic species described by Schmidt (1951) as "*Mercierella*", a junior synonym of *Ficopomatus*, are unlikely to belong to this genus (ten Hove and Weerdenburg, 1978: 101), and the Late Jurassic *Mercierella*(?) *dacica* Dragastan, 1966 is not a serpulid, but most likely a calcareous alga (*ibid*.).

Given that representatives of clade AI ("*Serpula*-group") have the most diverse and complex tube ultrastructures (Vinn et al., 2008b) and considering its intensive radiation during the Cenozoic, it is likely that the main ultrastructural diversification of serpulid tubes, which resulted in appearance of highly ordered ultrastructures, also took place at that time. This may partly explain why Mesozoic, especially Jurassic, serpulids do not show such ultrastructural diversity (e.g. Vinn and Furrer, 2008) as seen in Recent forms. On the contrary, ultrastructural diversity of Cenozoic material looks to be close to that of Recent taxa (Vinn, 2007). Species-level radiation within extant genera of serpulid clade AII ("*Spirobranchus*-group") also could have happened largely during the Cenozoic, while most genera seem to be of Mesozoic origin.

Recent diversity, which counts around 500 species, is not necessary indicative of intensive diversification in evolution of Serpulidae during Pleistocene-Holocene (2.6 Ma to Recent). Because the fossil record is never as well-known as Recent diversity, comparing Recent richness with generalised numbers for large time intervals covering millions of years is speculative. Numerous Recent species identifiable by their tube morphology and geographic distribution have been recognised in Pliocene-Holocene sediments (Table 1) (e.g. Di Geronimo and Sanfilippo, 1992).

3.9. Calcareous sabellids: rise and fall during the Mesozoic-Cenozoic

Calcified sabellids of the genus *Glomerula* appeared during the Late Paleozoic (Late Carboniferous, see above) or Early Jurassic (Late Hettangian; 200 Ma) and flourished in Mesozoic shallow seas producing numerous species (Jäger, 2005: Table 1), which were amongst the most common encrusters in Mesozoic shallow-water serpulid communities all over the world, often constituting up to 50% of total number of tubes. They were so common that six out of seven known Mesozoic sabellid species were described already in the early 19th century by pioneers of paleontology (von Schlotheim, 1820; Defrance, 1827b; J. de C. Sowerby, 1829; Goldfuss, 1831). Besides typical forms, the diversity of fossil Mesozoic *Glomerula* includes pseudocolonial species appearing as large irregular glomerates of interweaving tubes (fig. 7E), and species with strange internal tube structures making the lumen cross-section triradial (Jäger, 1983; 1993; 2005; see fig. 8A). Late Cretaceous sabellids demonstrate "balls-of-wool" tube coiling with no visible attachment areas, probably indicating a transition to the "rolling stone" lifestyle (Savazzi, 1999). Gradual decrease in abundance of calcareous sabellids relative to that of serpulids during the subsequent Cenozoic suggests that more advanced biomineralisation system acquired by serpulids allowed greater evolutionary plasticity of coiling and growth modes, thus giving serpulids competitive advantage over sabellids. The most crucial competitor for sabellids was probably *Hydroides*, which spread widely over shallow-water environments when calcareous sabellids declined. However, precise timing of this change is unclear because during the Oligocene (34-23 Ma) neither *Hydroides*, nor *Glomerula* seem to be common.

3.10. "False serpulids" of the Cenozoic: a fossil record bias

As in the Paleozoic, the outline of Cenozoic serpulid history is somewhat disturbed by numerous records of false serpulids as well as some true serpulids described as belonging to different fossil groups. Two examples are tusk-shaped scaphopods, which are often confused with serpulid genus *Ditrupa*, and vermetid gastropods, which have irregularly coiled shells with complex sculpture comparable to that of *Spirobranchus* tubes. Shells of both these mollusc groups are frequently confused with serpulid tubes in older zoological publications and even in current zoological practice (ten Hove, 1994). Therefore, numerous fossils described as "*Dentalium*" or "*Ditrupa*" in older publications need to be re-investigated (as e.g. done by Palmer, 2001). Scaphopods are an ancient group first appearing in the Paleozoic, while tusk-shaped serpulid worms with circular cross-section (*Ditrupa*) appear only in the latest Mesozoic. This means that for most of the Mesozoic the tusk-shaped serpulids are easily distinguishable from scaphopods by multiangular cross-sections of the tube. Confusion of serpulids with vermetids (e.g. part of species in Zelinskaja, 1962) is typical mainly for the material from Paleogene and Neogene periods, when irregularly coiled gastropods became common. There are also few records of problematic fossils from the Cenozoic, e.g. phosphatic tubes from the Paleogene of Chile described as serpulid *Semiserpula chilensis* by Wetzel (1957). Because phosphate mineralogy is unknown for Recent serpulids, the affinity of these irregularly loop-coiled tubes remains unclear.

3.11. Serpulid reefs and sediments

In Recent ecosystems, serpulid tubes contribute to sediment and reef formation (reviewed by ten Hove and van den Hurk, 1993 and Ferrero et al., 2005). *Serpula vermicularis* Linnaeus, 1758 and *Galeolaria hystrix* Mörch, 1863 build reefs in temperate seas with normal salinity (ten Hove and van den Hurk, 1993), while extensive reefs of *F. enigmaticus* (Fauvel, 1923) are found in brackish-water subtropical locations around the world (Dittmann et al., 2009). Tubes of free-lying Recent *Ditrupa* form shell banks (density up to 1000 ind. m^{-2}) on continental shelves in temperate to tropical seas all over the

world (ten Hove and van den Hurk, 1993), and *D. arietina* (O. F. Müller, 1776) significantly contributes to calcite sediment production in temperate seas (Medernach et al., 2000). Both serpulid reefs and banks produced by free-lying forms are known in the fossil record.

The "serpulid" reefs from Paleozoic sediments were formed not by true annelids, but by tentaculitoids, the group closely related to lophophorates (Vinn and Mutvei, 2009). The earliest true serpulid build-ups are known from the Late Triassic (Norian) of Europe (ten Hove and van den Hurk, 1993; Berra and Jadoul, 1996; Cirilli et al., 1999), around the Triassic-Liassic boundary in Spain (Braga and López-López, 1989), and Middle Jurassic of Southeastern Spain (Navarro et al., 2008). They became common in the Late Jurassic-Early Cretaceous (Regenhardt, 1964; Palma and Angeleri, 1992; ten Hove and van den Hurk, 1993; Kiessling et al., 2006). "*Serpula*" *coacervata* Blumenbach, 1803 tube fragments form a considerable portion of the rock mass around the Jurassic/Cretaceous boundary in north Germany (ten Hove and van den Hurk, 1993), probably being restricted to brackish water environments, the formation of such rocks may be explained by wave erosion of some build-ups. In younger Cenozoic rocks serpulid build-ups are described from the Early Eocene deposits of India (Ghosh, 1987), Miocene and Pliocene of Spain (ten Hove and van den Hurk, 1993), and Miocene (23-5 Ma) of the southern part of Eastern Europe (south Poland, Ukraine, Moldova). Miocene deposits of Eastern Europe contain especially numerous spirorbin and serpulid build-ups (Pisera, 1996; Górka et al., 2012), and the mass occurrence of serpulid build-ups is explained by enormously high water alkalinity in isolated water basins of the Paratethys (Górka et al., 2012). The diversity of serpulids constituting these reefs has not been studied, and at least some of these "serpulids" can be vermetid gastopods (see section 3.10). Sub-recent records of serpulid reefs include those from the Mid-Holocene of Argentina (Ferrero et al., 2005) and the Holocene of California (Howell et al., 1937).

Fossil banks of free-lying serpulids are known from the latest Early Jurassic (Late Toarcian; 176 Ma) of England, Middle Jurassic of Germany and France (Jäger, unpubl.); Middle Jurassic (Bathonian; ~167 Ma and Late Callovian; ~164 Ma) in Crimea (Ippolitov, unpubl.). In all listed cases banks are formed by mass occurrence of tetragonal spirally coiled *Nogrobs s. str.* tubes. Banks formed by tusk-shaped *Ditrupa*, similar to those known from Recent seas, become common from earliest Paleogene (Danian; 66 Ma) onwards in Europe (Jäger, unpubl.), and are also described from the Early Miocene (~20 Ma) of Taiwan (Cheng, 1974).

Both banks and carbonate build-ups in fossil state result in carbonate rocks consisting mainly of serpulid tubes with some matrix, called "serpulit" (alternatively, "serpula limestone" or "spirorbis limestone") by geologists.

3.12. Serpulids in deep-sea chemosynthetic communities

Serpulids apparently colonised seeps during the Jurassic: their first appearance in such environments is recorded from the latest Volgian (~146 Ma) of Svalbard (Vinn et al., in press). Fossil (Early Cretaceous) serpulid communities from methane seeps are characterised by low species diversity and mostly low abundance (Vinn et al., 2013). Hydrocarbon seep serpulids belong to several genera only (Vinn et al., 2013 and in press), and in the majority of fossil seeps only a single species was found. This pattern resembles that of molluscs from vents and seeps (Kiel, 2010a, 2010b). Unlike many gastropods and bivalves at vents and seeps that are restricted to these environments, serpulids are 'colonists' (Olu et al., 1996a): taxa from the surrounding sea floor that opportunistically invade seeps and vents because of the high abundance of organic matter. The fact that both serpulids and molluscs started colonising the seep environment shortly after their first appearance in the geological record supports the hypothesis that the seep faunas share evolutionary traits with the deep-sea fauna in general (Kiel and Little, 2006).

Similar to serpulids of fossil seeps, most serpulids at modern vents (ten Hove and Zibrowius, 1986; Kupriyanova et al., 2010) and seeps (Olu et al., 1996a, 1996b) also show low diversity. Seep serpulid abundance is high relative to the surrounding seafloor, but low to moderate compared to that of molluscs or siboglinid tubeworms that typically dominate these ecosystems (Vinn et al., 2013).

4. Conclusions and future studies: where to go next

Because studies of fossil serpulid tubes have no well-established stratigraphical, paleoecological or biogeographical application in palaeontology, the end result is that relatively little attention has been traditionally paid to the fossil record of this group. Concerted efforts of both palaeontologists and zoologists are required to advance our understanding of serpulid evolutionary history. Palaeontologists need to provide fossil material from poorly studied stratigraphical intervals (especially re-evaluation of problematic Late Paleozoic tubicolous fossils, the Early Cretaceous gap, and review of the Cenozoic fauna) and from poorly studied geographical regions (mainly outside Europe). New robust phylogenies with greater taxon coverage and integrating new molecular and morphological data from all serpulid genera are expected from zoologists. Further ultrastructural, mineralogical and histochemical studies of both Recent and fossil tubes are needed for reliable linking of fossils to Recent taxa.

Examination of genetic differences between closely related taxa allowing the estimation of a divergence time based on a known rate of accumulation of neutral genetic differences, known as molecular clock. No attempts have been made to age the Serpulidae based on genetic data, even though main diversification events can be roughly dated by the fossil record. This fossil record can provide an invaluable tool for calibration of molecular clocks not only in serpulids, but by extrapolation also in other annelid groups that lack a fossil record.

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Polychaete assemblages associated with the invasive green alga *Avrainvillea amadelpha* and surrounding bare sediment patches in Hawaii

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Abstract

Magalhães, W.F. and Bailey-Brock, J.H. 2014. Polychaete assemblages associated with the invasive green alga *Avrainvillea amadelpha* and surrounding bare sediment patches in Hawaii. *Memoirs of Museum Victoria* 71: 161–168.

Avrainvillea amadelpha is one of the most widespread invasive green algae on Hawaii's reefs, but virtually nothing is known about its associated fauna. A total of 16 sampling stations were selected: ten stations were distributed in areas where the invasive alga occurred abundantly ('A' stations) and six stations were placed on bare sand patches ('S' stations). Three replicates of ~475 cm³ each were collected in March 2010 at each station, by hand, using a nalgene corer (11 cm in diameter by 5 cm deep). Based on the comparison between *Avrainvillea amadelpha*-dominated bottoms and the surrounding bare sediment patches, our study demonstrates that these habitats support a diverse and abundant polychaete assemblage, with 2621 individuals and 84 species collected. The species *Sphaerosyllis densopapillata* (34.14%), *Phyllochaetopterus verrilli* (8.32%), *Protocirrineris mascaratus* (5.9%), *Exogone longicornis* (4.9%) and *Syllis cornuta* (4.47%) are the dominant taxa. The non-metric multidimensional scaling clearly separated the 'A' stations from the 'S' stations. ANOSIM has shown that stations within the *a priori*-defined group 'A' are significantly dissimilar from the stations in the group 'S' ($R = 0.527$; $P = 0.1\%$). SIMPER analysis has confirmed the slight but greater dissimilarity between algae and sediment stations (average dissimilarity = 60.12) than within either algae (52.27) or sediment stations (52.04). The invasive green alga *A. amadelpha* facilitates the development of above-ground polychaete communities, but the negative effects of this invader on the infaunal communities should be further investigated.

Keywords invasive species, seaweeds, *Avrainvillea amadelpha*, Polychaeta, Maunalua Bay

Introduction

Invasive species are considered to be one of the greatest threats to marine biodiversity (Vitousek et al., 1997) and can act by altering the physical environments in significant ways for other species (Cuddington and Hastings, 2004). Macroalgae are especially worrying invaders because they can develop into ecosystem engineers, changing food webs and spreading beyond their initial point of introduction efficiently (Thresher, 2000).

The effects of invasive algae on indigenous communities are being increasingly well-understood, particularly through studies concerning *Caulerpa* species. *Caulerpa taxifolia* has spread in temperate regions worldwide and modifies chemical and physical sediment and water properties (Gribben et al., 2013). The presence of this species is known to increase the density of epibiont organisms by facilitating recruitment of native species (Gribben and Wright, 2006; Bulleri et al., 2010). However, it may also decrease the density of infaunal organisms and modify the abiotic environment (Neira et al., 2005; Gribben et al., 2013). The presence of the invasive *Caulerpa racemosa* var. *cylindracea* in the Mediterranean has been proven to expand suitable habitat for polychaete worms, increasing the number of associated species (Argyrou et al., 1999; Box et al., 2010; Lorenti et al., 2011).

The green siphonous alga *Avrainvillea amadelpha* (Montagne) A. Gepp and E. Gepp, 1908 (Order Bryopsidales) has been reported since the early 1980s from the south-east shore of Oahu, Hawaii (Brostoff, 1989) and now is considered one of the most widespread invasive non-indigenous species in Maunalua Bay (Coles et al., 2002). This species proliferates in soft bottom habitats, co-occurring in areas that were once dominated by the endemic Hawaiian sea grass *Halophila hawaiiana* (Smith et al., 2002).

Efforts to remove introduced algae from reefs in Kaneohe Bay and off Waikiki have been ongoing and have achieved some success (Smith et al., 2004). However, little effort has

been made to investigate whether the invertebrate taxa inhabiting the bottoms dominated by these invasive algae are composed of native species or introduced species.

Avrainvillea amadelpha mats typically serve as substrates for many native species of epiphytic algae (Smith et al., 2002), and this association is known to increase the diversity of associated faunal assemblages by providing food and shelter (Johnson and Scheibling, 1987; Duffy, 1990). The physical complexity of the habitat may also be increased, providing a refuge from fish predation (Coull and Wells, 1983; Dean and Connell, 1987) and greater availability of surface area for recruitment (Connor and McCoy, 1979, McGuinness and Underwood, 1986). Algal turfs have been shown to reduce impact from wave exposure (Dommasnes, 1968) and enhance communities on exposed reefs (Bailey-Brock et al., 1980).

The macrobenthic assemblages associated with invasive algae in Hawaii are scarcely known, and this study aimed to provide baseline data on the polychaete worms associated with *A. amadelpha* mats and nearby bare sediment patches prior to removal efforts.

Materials and methods

Study area and sampling design

This study was carried out on nearshore reef flats in Maunalua Bay on the south shore of Oahu, Hawaii (fig. 1). The area is predominantly composed of consolidated limestone reef flats covered by a shallow layer of fine to coarse sand. The reef flats support abundant growth of the non-indigenous algae *Gracilaria salicornia, Hypnea musciformis* and *Avrainvillea amadelpha* (Coles et al., 2002).

A total of 16 sampling stations were selected for this study: ten stations were distributed in areas where *Avrainvillea amadelpha* occurs abundantly ('A' stations) and six stations were placed on bare sand patches ('S' stations; fig. 1). Three replicates of approximately 475 cm³ each were collected in March 2010 at each station, by hand, using a nalgene corer (11 cm in diameter by 5 cm deep). The *Avrainvillea amadelpha* samples ('A' stations) were composed of sediment to a depth of 5 cm and the overlying algae within the corer. The sediment samples ('S' stations) consisted of the top 5 cm of sediment.

All samples were fixed in buffered 4% formalin and Rose Bengal mixture immediately after sampling for a minimum of 48 h. Organisms were carefully removed from the crevices and branches of the algae, placed in 70% ethanol, then the sediments were elutriated over a 0.5-mm sieve and the retained infauna placed in 70% ethanol. The polychaetes were sorted, counted and identified to the lowest taxonomic level possible using compound and dissecting microscopes.

For comparative purposes, 20 samples of *Gracilaria salicornia* were collected (ten samples during the summer months and ten during the winter months of 2009) on the reef in front of the Natatorium in Waikiki on the south shore of Oahu, Hawaii. Samples were collected with a nalgene corer (11 cm in diameter by 5 cm deep). This dataset is part of an unpublished report by C. Moody, and the samples were donated and the polychaetes were later identified to species level by the authors.

Data analyses

The replicates within each station were summed, and the abundance (*N*), species richness (*S*), and Shannon–Wiener diversity index (log e; *H'*), and Pielou's Evenness (*J'*) were calculated for each station. nMDS ordination was constructed to produce two-dimensional ordination plots to show relationships between stations in relation to polychaete abundance and richness.

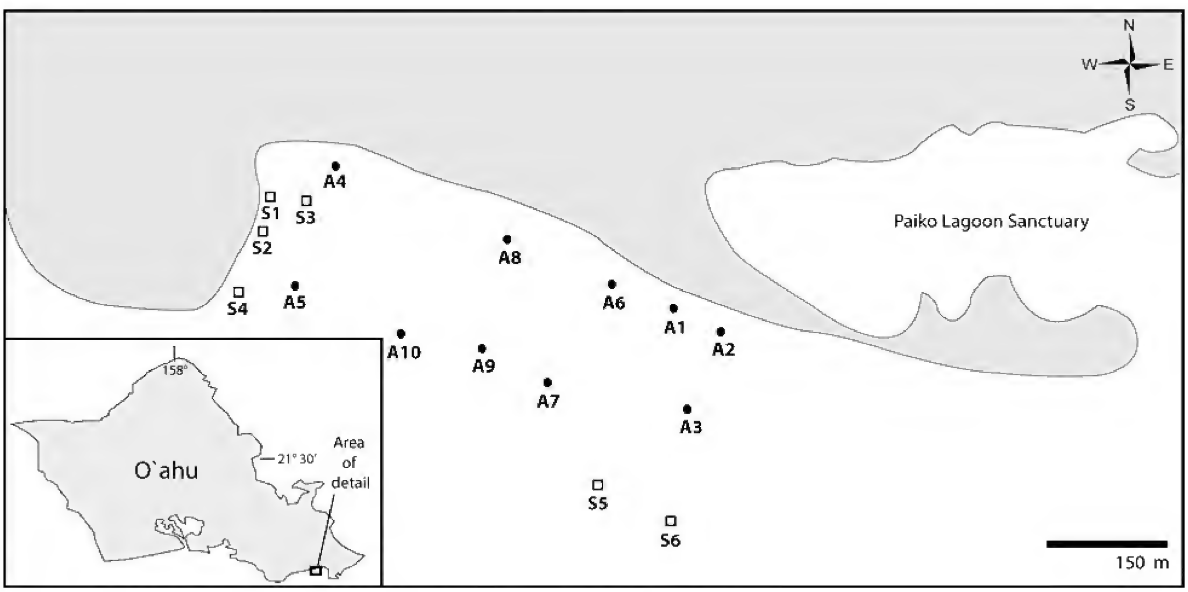

Figure 1. Map of the study area showing the algae ('A' stations; circles) and sediment stations ('S' stations; squares).

Table 1. Summary of the results from SIMPER analysis with the species that contributed to up to 60% of the similarity within each group of stations.

Species/contribution to similarity (up to 60%)	**'A' stations (%)**	**'S' stations (%)**
Sphaerosyllis densopapillata (Syllidae)	14.84	16.89
Exogone verugera (Syllidae)	8.34	
Branchiosyllis exilis (Syllidae)	7.07	
Armandia intermedia (Opheliidae)	6.63	
Lysidice nr. *unicornis* (Eunicidae)	6.19	
Scyphoproctus sp. (Capitellidae)	5.71	
Perinereis nigropunctata (Nereididae)	5.12	
Syllis cornuta (Syllidae)	4.92	8.86
Phyllodoce parva (Phyllodocidae)	4.71	
Lumbrineris dentata (Lumbrineridae)		10.12
Exogone longicornis (Syllidae)		9.01
Paraonella sp. (Paraonidae)		8.65
Westheidesyllis heterocirrata (Syllidae)		6.53

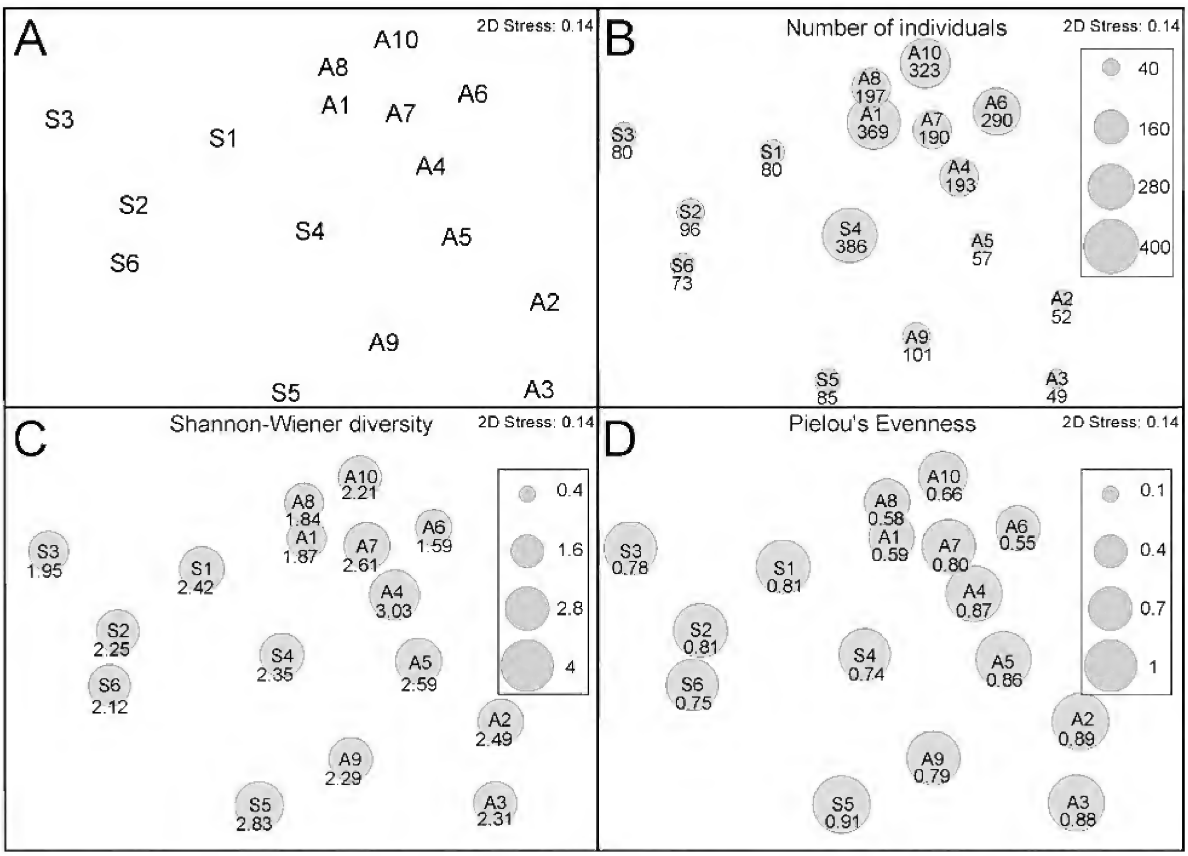

Figure 2. nMDS ordinations of polychaete assemblages: A, using data of all taxa; B, bubbles indicating abundance in number of individuals; C, bubbles indicating values of Shannon–Wiener diversity; D, bubbles indicating values of Pielou's Evenness.

An analysis of similarity (ANOSIM) was performed to test the statistical significance of the *a priori*-defined groups (i.e. 'A' stations *vs.* 'S' stations). Similarity percentage analysis (SIMPER) identified those taxa that explained relatively large proportions of the similarity within a group. All multivariate analyses were done using the Bray–Curtis similarity coefficient with non-standardized and fourth root transformed data using PRIMER 6.0 software.

Results and discussion

A total of 2621 polychaetes representing 84 taxa were collected. The 'A' stations had a total of 64 taxa and 1821 individuals, while the 'S' stations had a total of 47 taxa and 800 individuals. The most abundant species were *Sphaerosyllis densopapillata* (Syllidae; 34.14%), *Phyllochaetopterus verrilli* (Chaetopteridae; 8.31%), *Protocirrineris mascaratus* (Cirratulidae; 5.9%), *Exogone longicornis* (Syllidae; 4.9%) and *Syllis cornuta* (Syllidae; 4.47%). Syllid polychaetes comprised the most abundant and rich polychaete family, with 1517 individuals and 24 species. Syllids are known to be the most abundant and species-rich polychaete family associated with *Posidonia* beds in the Mediterranean (e.g. Gambi et al., 1995).

The non-metric multidimensional scaling clearly separated the 'A' stations from the 'S' stations (fig. 2). The Shannon–Weiner diversity index did not seem to explain many of the differences between the groups; however, polychaetes from the 'A' stations occurred in greater abundance compared with the 'S' stations, with exception of station S4 (fig. 2).

ANOSIM indicated that stations within the *a priori*-defined group 'A' were significantly dissimilar from stations in the group 'S' (R = 0.527; P = 0.1%). SIMPER analyses confirmed the slight but greater dissimilarity between algae and sediment stations (average dissimilarity = 60.12) than within either algae (52.27) or sediment stations (52.04). The syllid *Sphaerosyllis densopapillata* was the most abundant species overall and explained 14.82% of the similarity within the 'A' stations and 16.89% within the 'S' stations (table 1). The other top taxa varied greatly between the types of stations (table 1). *Sphaerosyllis densopapillata* was removed from the analysis of similarity to verify the influence of this abundant species on the dissimilarity between stations. The average dissimilarity increased between algae and sediment stations (from 60.12 to 64.5) and within both algae (from 52.27 to 55.6) and sediment stations (from 52.04 to 56).

Even though there were significant differences between the stations located on *Avrainvillea amadelpha* mats and those sampled on sediments without the algae (ANOSIM), stations within the algal mats were also dissimilar. This might have been explained if other variables such as length and density of algal branches, amount and size of the sediment within the branches, human disturbance near shore, and nature of the underlying substrate were measured.

Several polychaete worms, including the tube builder *Mesochaetopterus minutus* and the syllid *Westheidesyllis heterocirrata*, were predominantly collected from the bare sediment patches. *Mesochaetopterus minutus* is a gregarious worm that forms tufts of sand-covered tubes and is mainly found on shallow-water reef flats along O'ahu's south shore (Bailey-Brock, 1979, 1987). This species may be playing an important role in these assemblages by binding the sediments loosened by the algal removal efforts in and around their tubes. Chaetopterids can reach densities of 62,400 per m^2 on O'ahu's south shore, and if they are present in high densities on the outer reef flats of the area where *A. amadelpha* has been removed, they may retain the sediments that would otherwise be transported closer to the shore (Bailey-Brock, 1979). Chaetopterid mounds retain a high abundance of polychaetes but a low diversity, with only 22 species being found by Bailey-Brock (1979).

The diversity of polychaete species found in *Avrainvillea amadelpha* mats is considerably higher than that found in another invasive alga, *Gracilaria salicornia*, present in south Oahu (table 2). *Gracilaria salicornia* is low growing, less structured and has small thalli and many branches, as opposed to *A. amadelpha*. A total of 15 polychaete species have been found commonly in both invasive algae, and the syllids were the dominant family in *G. salicornia* as well (table 2). The most abundant polychaetes associated with *G. salicornia* were *Nereis jacksoni*, *Syllis cornuta* and the ctenodrilid *Raphidrilus hawaiiensis*. The ctenodrilid was originally described from those algal assemblages (Magalhães et al., 2011) and has been found in low abundance in association with *A. amadelpha* at the study site.

Avrainvillea amadelpha mats are a suitable habitat for polychaetes at this location, especially for those detritus feeders favoured by the fine sediment coating accumulated on the branches and in crevices of the alga. The presence of the macroalga *Caulerpa racemosa* has also been shown to increase the diversity and abundance of polychaetes (Argyrou et al., 1999; Box et al., 2010). Lorenti et al. (2011) also observed that polychaetes increased in percentage contribution to the total macrofaunal diversity after the introduction of *C. racemosa*.

The development of above-ground communities are usually facilitated by the presence of invasive macroalgae because of the added structure they give to previously unstructured habitats (Wonham et al., 2005; Gribben et al., 2013). This unnatural increase in diversity in previously unvegetated sediments may have detrimental effects, especially below ground. For instance, the biomass of the invasive *Caulerpa taxifolia* has been negatively associated with the abundance of infaunal organisms (Gribben et al., 2013), and modification of environmental parameters below ground by invasive species has also been noted (e.g. Neira et al., 2005).

Current efforts to remove the attached *A. amadelpha* in the area of this study may help to recover the previous ecological state of the local polychaete assemblages, since infaunal organisms have been known to recover quickly after restoration of the sedimentological characteristics of the habitat (Dernie et al., 2003). Further collections after the removal efforts will be necessary to compare with the results presented herein. This study was conducted two months before the first effort at removal of invasive alga from the area and represents important baseline information for understanding the resilience of this ecosystem.

Table 2. Taxonomic list of polychaete species organized by family found on bare sediment patches, *Avrainvillea amadelpha* and from another invasive alga *Gracilaria salicornia*.

	Avrainvillea amadelpha	Bare sediments	*Gracilaria salicornia*
Amphinomidae			
Eurythoe sp.	X	X	
Linopherus microcephala (Fauvel, 1932)		X	
Ampharetidae			
Lysippe sp.	X		
Capitellidae			
Capitella jonesi (Hartman, 1959)	X		
Capitellethus cinctus Thomassin, 1970	X		
Heteromastus cf. *filiformis* (Claparède, 1864)	X		
Notomastus tenuis Moore, 1909		X	
Scyphoproctus pullielloides Hartmann-Schröder, 1965	X	X	
Scyphoproctus sp.	X	X	
Chaetopteridae			
Mesochaetopterus minutus Potts, 1914		X	
Phyllochaetopterus verrilli Treadwell, 1943	X	X	X
Cirratulidae			
Aphelochaeta sp.	X		
Caulleriella bioculata (Keferstein, 1862)	X		
Caulleriella sp.	X		
Cirriformia crassicollis (Kinberg, 1866)	X	X	
Cirriformia sp.	X		
Monticellina nr. *cryptica* Blake, 1996	X		
Protocirrineris mascaratus Magalhães & Bailey-Brock, 2013	X	X	
Tharyx sp.	X		
Timarete hawaiensis (Hartman, 1956)			X
Timarete punctata (Grube, 1859)			X
Cossuridae			
Cossura cf. *coasta* Kitamori, 1960	X		
Ctenodrilidae			
Raphidrilus hawaiiensis Magalhães, Bailey-Brock & Davenport, 2010	X		X
Dorvilleidae			
Dorvillea sp.	X		X
Protodorvillea biarticulata Day, 1963		X	
Eunicidae			
Eunice afra Peters, 1854	X	X	
Eunice antennata (Savigny in Lamarck, 1818)			X
Lysidice nr. *ninetta* Audouin & Milne-Edwards, 1833	X		
Lysidice nr. *unicornis* (Grube, 1840)	X	X	X
Flabelligeridae			
Flabelligeridae gen. sp.	X		

	Avrainvillea amadelpha	Bare sediments	*Gracilaria salicornia*
Hesionidae			
Hesionidae fragment			X
Lumbrineridae			
Lumbrineris dentata Hartmann-Schröder, 1965	X	X	X
Lumbrineris latreilli Audouin & Milne Edwards, 1834		X	
Maldanidae			
Axiothella quadrimaculata Augener, 1914			X
Rhodine sp.	X		
Nereididae			
Micronereis sp.	X		
Neanthes arenaceodentata (Moore, 1903)		X	
Nereis jacksoni Kinberg, 1866			X
Nereis sp.	X		
Perinereis nigropunctata (Horst, 1889)	X	X	
Platynereis dumerilii (Audouin & Milne Edwards, 1834)			X
Oenonidae			
Arabella sp.	X		
Arabella iricolor (Montagu, 1804)	X	X	
Opheliidae			
Armandia intermedia Fauvel, 1902	X	X	
Polyophthalmus pictus (Dujardin, 1839)	X		
Orbiniidae			
Naineris sp.	X	X	
Questa caudicirra Hartman, 1966		X	
Questa retrospermatica Giere, Ebbe and Erseus, 2007	X	X	
Oweniidae			
Galathowenia oculata (Zachs, 1923)	X	X	
Paraonidae			
Aricidea sp.	X		
Cirrophorus sp.		X	
Paraonella sp.	X	X	
Phyllodocidae			
Eumida sp.		X	X
Phyllodoce parva (Hartmann-Schröder, 1965)	X	X	
Pilargidae			
Synelmis cf. *gibbsi* Salazar-Vallejo, 2003		X	
Protodrilidae			
Protodrilus albicans Jouin, 1970		X	
Sabellidae			
Amphiglena mediterranea (Leydig, 1851)	X	X	
Branchiomma nigromaculatum (Baird, 1865)	X		X
Euchone sp.	X		

	Avrainvillea amadelpha	Bare sediments	*Gracilaria salicornia*
Sigalionidae			
Sigalionidae gen. sp.		X	
Spionidae			
Aonides sp.	X		
Laonice nr. *cirrata* (M. Sars, 1851)	X		
Microspio granulata Blake and Kudenov, 1978		X	X
Spio filicornis (Müller, 1776)		X	X
Sternaspidae			
Sternaspis sp.	X		
Syllidae			
Branchiosyllis exilis (Gravier, 1900)	X	X	
Brania rhopalophora (Ehlers, 1897)	X	X	X
Brania sp.	X		X
Exogone longicornis Westheide, 1974	X	X	
Exogone sp.	X	X	X
Exogone verugera (Claparède, 1868)	X	X	X
Grubeosyllis mediodentata (Westheide, 1974)	X		
Haplosyllis sp.	X	X	X
Myrianida pachycera (Augener, 1913)	X		X
Odontosyllis sp.	X		
Opistosyllis sp.		X	
Pionosyllis sp.		X	
Sphaerosyllis centroamericana Hartmann-Schröder, 1974			X
Sphaerosyllis densopapillata Hartmann-Schröder, 1979	X	X	
Sphaerosyllis riseri Perkins, 1981	X		
Sphaerosyllis sp.	X		
Syllinae juv.		X	
Syllis cornuta Rathke, 1843	X	X	X
Syllis variegata Grube, 1860	X		
Trypanosyllis sp.	X		
Typosyllis aciculata orientalis Imajima & Hartman, 1964	X	X	
Typosyllis crassicirrata Treadwell, 1925			X
Typosyllis ornata Hartmann-Schröder, 1965		X	
Typosyllis sp.	X		X
Virchowia japonica Imajima & Hartman, 1964	X		
Westheidesyllis heterocirrata (Hartmann-Schröder, 1959)	X	X	
Terebellidae			
Nicolea gracilibranchis (Grube, 1878)			X
Polycirrus sp.	X		X
Trichobranchidae			
Trichobranchus nr. *glacialis* Malmgren, 1866	X		

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Incipient speciation within the *Namalycastis abiuma* (Annelida: Nereididae) species group from southern India revealed by combined morphological and molecular data

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Abstract

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Namalycastis abiuma (Grube, 1872), originally described from Brazil, comprises a species complex of morphologically similar forms occurring circumtropically, including India. Apart from the *Namalycastis abiuma* species group, four other *Namalycastis* species are presently known from India: *N. indica* Southern, 1921, *N. fauveli* Nageswara Rao, 1981, *N. glasbyi* Fernando & Rajasekaran, 2007, and *N. jaya* Magesh, Kvist & Glasby, 2012. Recent sampling along the southern Indian coast has uncovered new specimens of the *N. abiuma* species group. The present study uses combined morphological and molecular data (DNA barcoding) to explore species boundaries within the complex in southwest India and thereby resolve existing taxonomic confusion. In order to evaluate morphological variability within the *N. abiuma* species group, a total of 50 specimens were sampled from different geographical regions in southern India, and assessed using traditional methods. For 18 of the specimens, a 509 bp fragment of COI, the proposed DNA barcoding gene, was sequenced and subjected to tree reconstruction using both distance methods and maximum parsimony. Based on similarity alone, six different haplotypes were recognized within the dataset and these were also subsequently recovered as six distinct clades in the parsimony analysis. There is significant concordance between the morphotypes and the genetic haplotypes, suggesting that significant structural forces are acting on the specimens at a population level, and that these specimens may even be in an early stage of speciation.

Keywords Nereididae; Namanereidinae; *Namalycastis abiuma*; Taxonomy; Phylogeny; Genetic variation; DNA barcoding.

Introduction

The subfamily Namanereidinae (Nereididae) consists of three genera (*Namalycastis* Hartman, 1959; *Namanereis* Chamberlin, 1919; and *Lycastoides* Johnson, 1903) and is most prominently known from the tropics and subtropics. Thirty-nine namanereidin species have been reported throughout the world and some of these pertain to larger complexes of problematic species, herein termed "species groups". The genus *Namalycastis* contains 22 species (Glasby 1999a, 1999b; Magesh et al. 2012; Conde-Vela 2013), four of which have so far been recorded on the Indian subcontinent. These include *N. indica* Southern, 1921, *N. fauveli* Nageswara Rao, 1981, *N. glasbyi* Fernando & Rajasekaran, 2007, and *N. jaya* Magesh, Kvist & Glasby, 2012. In addition, there are several records of the *N. abiuma* species group in southern India (Glasby 1999a; Magesh et al. 2012).

The *N. abiuma* species group concept was introduced by Glasby (1999a) for a group of individuals that 'exhibit a greater amount of morphological variation over their range than is typical for a namanereidinae species' (Glasby 1999a: 115). It was expected that such species groups 'will probably be found to contain more than one species with further characterisation of reproductive mode and genetics' (P. 115); that is, species groups likely comprise morphologically cryptic species. Indeed the first cryptic species, *N. jaya,* was discovered recently (Magesh et al. 2012). Cryptic species are recognised

when the species group hypothesis of Glasby (1999) is falsified by independent data, which in the present study is the DNA barcode gene. Our concept of species most closely follows the Synapomorphic Species Concept as defined by Wilkins (2003: 635): 'A species is a lineage separated from other lineages by causal differences in synapomorphies'.

Recognition of the *Namalycastis abiuma* species group follows Glasby (1999a). It has a noticeably broad diagnosis, but may be distinguished from many other *Namalycastis* species by having brown epidermal pigment on the dorsal side of the body (including the pygidium), short antennae (not extending beyond the tip of the palpophore) and coarsely serrated spinigerous chaetae (but not falcigerous chaetae) in parapodia of the posterior part of the body. The concept includes several separately described species including *Lycastis meraukensis* Horst, 1918 (described from New Guinea), *L. nipae* Pflugfelder, 1933 (Sumatra), *L. vivax* Pflugfelder, 1933 (Sumatra), *Namalycastis rigida* Pillai 1965 (Philippines) and *N. meraukensis var. zeylancia* (Sri Lanka) (Glasby 1999). Although the association of these species with the *N. abiuma* species group in Glasby (1999a) was intended to represent formal synonymy, the fact that the ICZN rules do not apply to species groups, means that all of these names, with the exception of the variety *N. meraukensis var. zeylancia* (also not covered by ICZN), are currently valid and potentially available to newly discovered cryptic species.

The most widely reported *Namalycastis* species in India, *N. indica,* is very similar to the *N. abiuma* species group in external appearance, and unless chaetal types and distributions are examined carefully, the two species are very difficult to separate. Most descriptions of *N. indica,* in the taxonomic literature fail to give an adequate account of chaetal types and distributions and it is therefore quite possible that the two species have been extensively confused (Glasby 1999). Doubtful taxonomic references to *N. indica* include those of Ghosh (1963), Day (1967), and Sunder Raj & Raj (1987). Because of this potentially wide confusion of specimens pertaining to the *N. abiuma* species group and the morphological similarity with *N. indica*, the addition of molecular tools for separation of species is becoming increasingly pressing. Such tools, if applied correctly, would enable taxonomists to both evaluate synonymous taxa and to separate this species complex into distinct taxa. Thus, studying the genetic variations within these species groups is important for inferring solid species diagnoses and in identifying potentially novel species, as well as addressing the question of how many species (*sensu* Wilkins, 2003) are present within these species groups. Here, we shed some light on part of this issue by examining specimens that are morphologically compatible with *N. abiuma* from different regions across the Indian subcontinent, and use both molecular and morphological techniques to clarify taxonomic ambiguity.

Materials and methods

Specimen collection

Between January 2008 and December 2009, polychaete worms were collected from various localities, at varying salinities and depths, along the southern Indian coast; Kadinamkulam Lake (depth 2 m), Kayamkulam Kayal (2-3 m), Cochin (Kochi) estuarine system (3 m), Thoothukudi mangroves (Tamilnadu; 1 m) and the Ariankuppam estuary of Puducherry (0.5 m) (Fig. 1). The sites were selected based on the habitat suitability and the presumed presence of *Namalycastis* spp. For the better part, specimens were collected from muddy sediments; at Kadinamkulam Lake, mud was commonly mixed with slightly rotting organic matter. All specimens have been deposited in the Zoological Reference Collection of the Zoological Survey of India, Kozhokode, Kerala, India.

Morphological examinations

Sampling strategies and identifications followed the method of Glasby (1999a) and Magesh et al. (2012). Descriptions are based on the same character set used by Glasby (1999a). After securing tissue for DNA extraction (see below), specimens were relaxed in isotonic $MgCl_2$, quickly submerged in 95% ethanol to evert the proboscis, fixed in 10% formalin and subsequently transferred to 70% ethanol. Fixed specimens were then dissected and the parapodia were mounted in polyvinyl lactophenol on microscope slides to enable microscopical examinations of the morphology. Tissues to be used for DNA sequencing were fixed in 95% ethanol and their further processing is described below.

DNA sequencing and analyses

A total of 18 specimens, identified as the *N. abiuma* species group were chosen for the molecular portion of this study and five specimens, including *N. jaya* and *Platynereis bicanaliculata* (Baird, 1863) were used as outgroup taxa; the trees were rooted with *P. bicanaliculata*. A complete list of specimens, sampling sites, and GenBank accession numbers can be found in Table 1.

Approximately 20-40 chaetigers of the posterior part of the worms (excluding the pygidium) were used for DNA extraction. Total genomic DNA was isolated from the specimens following the extraction protocol of Miller et al. (1988). Partial sequences of cytochrome *c* oxidase subunit I (COI) were PCR-amplified using the primers suggested by Ivanova et al. (2007) (i.e., FR1d [5'- TTCTCCACCAACCACAARGAYATYGG-3'] and FR1d_t1 [5'- CACCTCAGGGTGTCCGAARAAYCARAA-3']). The PCR used 30 cycles of the following protocol: an initial 5 minute denaturation step at 94°C for all samples, followed by 30 seconds denaturation at 94°C, 30 seconds annealing at 55°C, 2 minutes extension at 72°C and a final 5 minute extension step at 72°C for all samples. PCR products were subsequently checked on a 2% agarose gel and successful amplifications were gel eluted using a chromous gel extraction kit (Gel Extraction SPIN-50, Chromous Biotech, Bangalore, India) following the instructions given by the manufacturer. The DNA was then purified using a PureFast Genomic DNA purification kit (Helini Biomolecules, Chennai, India), cycle sequencing was carried out using the same primers as above, and ethanol precipitation prepared the DNA for sequencing. Nucleotide sequencing was then performed using an ABI 3500 XL Genetic Analyzer (Applied Biosystems, Foster City, CA). All nucleotide sequences are deposited at NCBI; accession numbers are presented in Table 1.

Assembly of forward and reverse strand sequences was carried out using BioEdit ver. 7.0.5.2 (Hall 1999), and reconciled sequences were aligned using MAFFT ver. 7 (Katoh & Standley

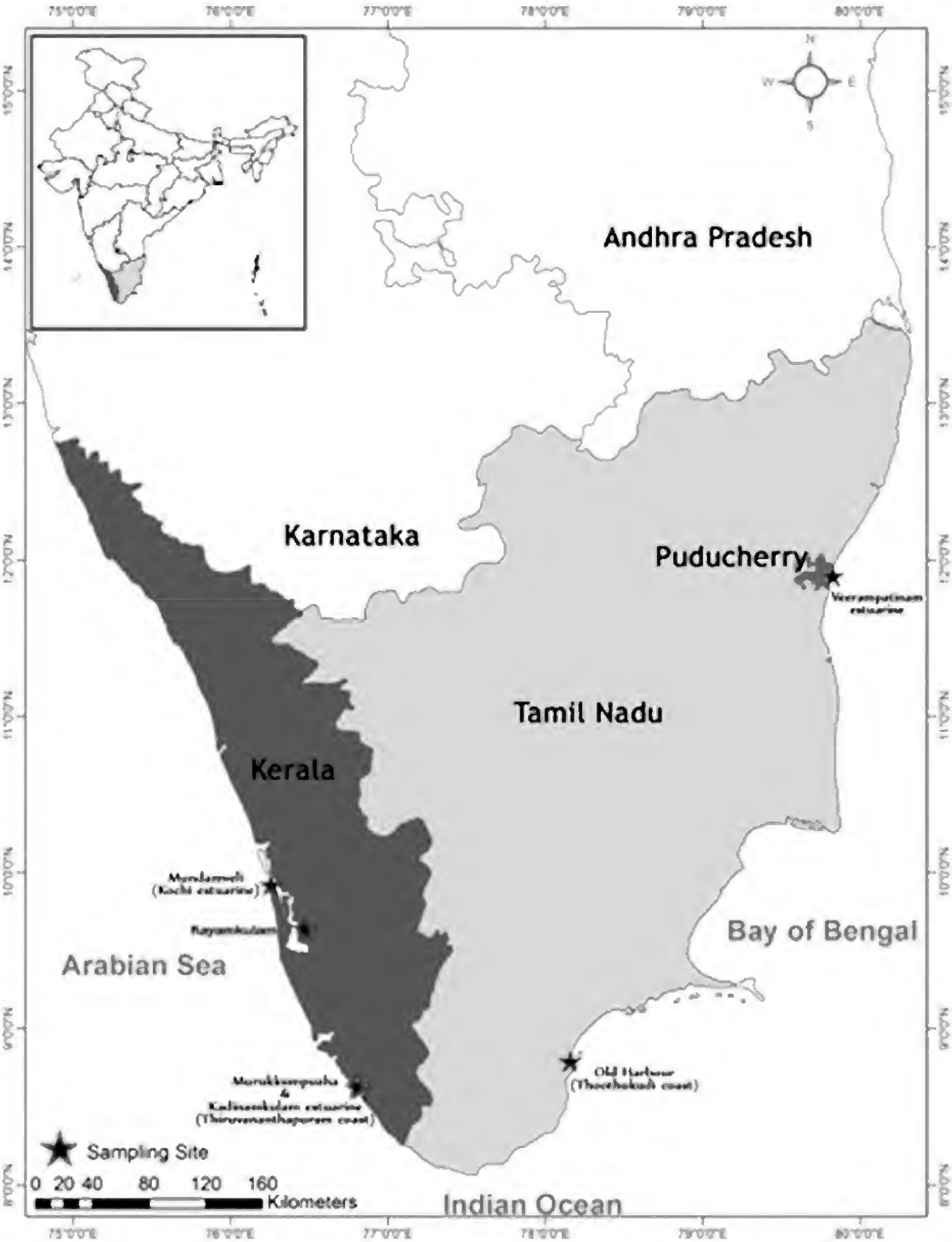

Figure 1. Map of the collection localities for the specimens of the *Namalycastis abiuma* species group.

2013) applying the L-INS-i strategy and default settings; note that the final alignment was devoid of gaps such that the sequences can be treated as pre-aligned. Intraspecific and interspecific variations were then calculated using MEGA ver. 5.2.2 (Tamura et al. 2011) with the following settings: uncorrected-p distances, pairwise deletion of gaps and using 1st, 2nd and 3rd codon positions. A Neighbour-Joining tree was constructed in PAUP* ver. 2.0b10 (Swofford 2002) using uncorrected-p distances. Also, a phylogenetic tree under the criterion of maximum parsimony was constructed using PAUP, where a heuristic search was performed employing 1000 initial addition sequences and TBR branch swapping. Support values for the nodes were estimated through bootstrap resampling using 100 random addition sequences and the same settings as above.

Results

Morphological analyses

In total, 50 specimens were collected that were identified to the *Namalycastis abiuma* species group. These specimens all fit within the range of the known intraspecific variation of the species, as follows:

Diagnosis. Brown epidermal pigment dorsally and on pygidium; prostomium with shallowly cleft anteriorly, antennae extending short of tip of palpophore; jaws with a single robust terminal tooth , 4-5 subterminal teeth, 3-5 teeth ensheathed proximally; notochaetae present or absent; neurochaetae arrangement Type A sensu Glasby (1999a); subneuroacicular falcigers in parapodia of chaetiger 10 with blades 4.3-5.7× longer than width of shaft head and having 4-15 fine to moderate sized teeth; subneuroacicular falcigers in parapodia of chaetiger 10 with blades 3.7-7.2 x longer than width of shaft head, up to 18 teeth; subneuroacicular spinigers in parapodia of posterior body with blades coarsely serrated proximally; heterogomph chaetae with boss not prolonged; pygidium with multi-incised rim (modified slightly after Glasby 1999a).

Preliminary morphological investigations suggested that the specimens can be further subdivided into two morphologically distinct lineages (subgroup 1 and 2). The morphological and some ecological characteristics of the subgroups are further discussed below. Within subgroup 1, a total of four morphotypes could be identified and within subgroup 2, an additional two morphotypes were found for a total of six morphotypes among the 50 specimens (Table 1).

Subgroup 1

Thirty-seven out of the 50 specimens were categorized as subgroup 1 on the basis of morphological characters.

Diagnosis: as for the *N. abiuma* species group except body uniform in width anteriorly, tapering gradually posteriorly. Eyes, 2 pairs, black, arranged obliquely, unequal in size, posterior pair larger than anterior pair. Posterodorsal tentacular cirri short and extending posteriorly to end of first chaetiger. Jaws with, 6 or 7 subterminal teeth and 4 teeth unsheathed proximally. One or two notochaetae per notopodium. Notopodial sesquigomph spinigers observed from chaetigers 5-11 until mid body; spinigers absent in posterior part of body. Supra-neuroacicular sesquigomph spinigers in chaetiger 10. Supra-neuroacicular falcigers in chaetiger 10 moderately serrated and teeth non-uniform in length. Sub-neuroacicular spinigers in chaetiger 10 with medium or finely serrated blades; blades with coarse serrations proximally posteriorly from chaetiger 30-120.

Intraspecific variation among morphotypes

M1. Specimens AQPA1- AQPA 10: Notochaetae present from chaetiger 8 to mid body, one or two per notopodium, in no particular order. Jaws with 11 teeth (Fig. 2A).

M2. Specimens AQMM1- AQMM10; AQMM51 – AQMM55: From fifth chaetiger to mid body, notochaetae single (rarely two; e.g. AQMM51) or absent in many mid-body parapodia. Jaws with 9 teeth (Fig. 2C). Number of homogomph spinigers usually greater than number of heterogomph chaetae; only subneuroacicular spinigers present in some chaetigers (e.g. chaetiger 18).

M3.Specimens AQMM82 & AQMM92: Notochaetae single or absent in posterior podia. Jaws with 10 teeth (Fig. 2B).

M4. Specimens AQMM6 and AQMM61-AQMM65: Parapodium and dorsal cirrus very wide in middle and posterior chaetigers (Fig. 2K). Dorsal cirri increase in length posteriorly (Fig. 2J).

Subgroup 2

Thirteen out of the 50 specimens were categorized as species group 2 on the basis of morphological characters.

Diagnosis: as for *N. abiuma* species group except, entire body with width tapering posteriorly. Antennae extending to tip of palpophore. Eyes equal or unequal in size (with posterior pair smaller); sometimes faded or absent.

Posterodorsal tentacular cirri long and extending posteriorly up to chaetiger 4 or 5. Jaws with 8 teeth (Fig. 2D). Notochaetae present from chaetiger 10-12. Supra-neuroacicular falcigers in chaetiger 10 with blades moderately or coarsely serrated, about 11 teeth. Sub-neuroacicular falcigers in chaetiger 12 with 14 teeth. Sub-neuroacicular spinigers in anterior body with blades finely serrated and sub-neuroacicular spinigers in posterior body with coarsely serrated blades.

Intraspecific variation among morphotypes

M5. Specimens K1-K10; K51-K55: Eyes are faded in a few specimens (e.g. K1, K3 and K53), absent in a few specimens (e.g. K5-10), and merged in a few specimens (Figs. 2I, 2E and 2F, respectively).

M6. Specimens K24 and K242: Three eyes present and about equal in size (Fig. 2G, H). Three acicula (rather than the usual two) present in chaetiger 10; Figs. 2L and 2M).

DNA barcoding and Neighbour-Joining

A total of 18 specimens were successfully sequenced for a 509 bp region of COI, representing all of the six morphotypes specified above (M1-M4 in species group 1, and M1-M2 in species group 2). In addition, COI sequences from *Namalycastis jaya* (HQ456363 and JN790065-67) and *Platynereis*

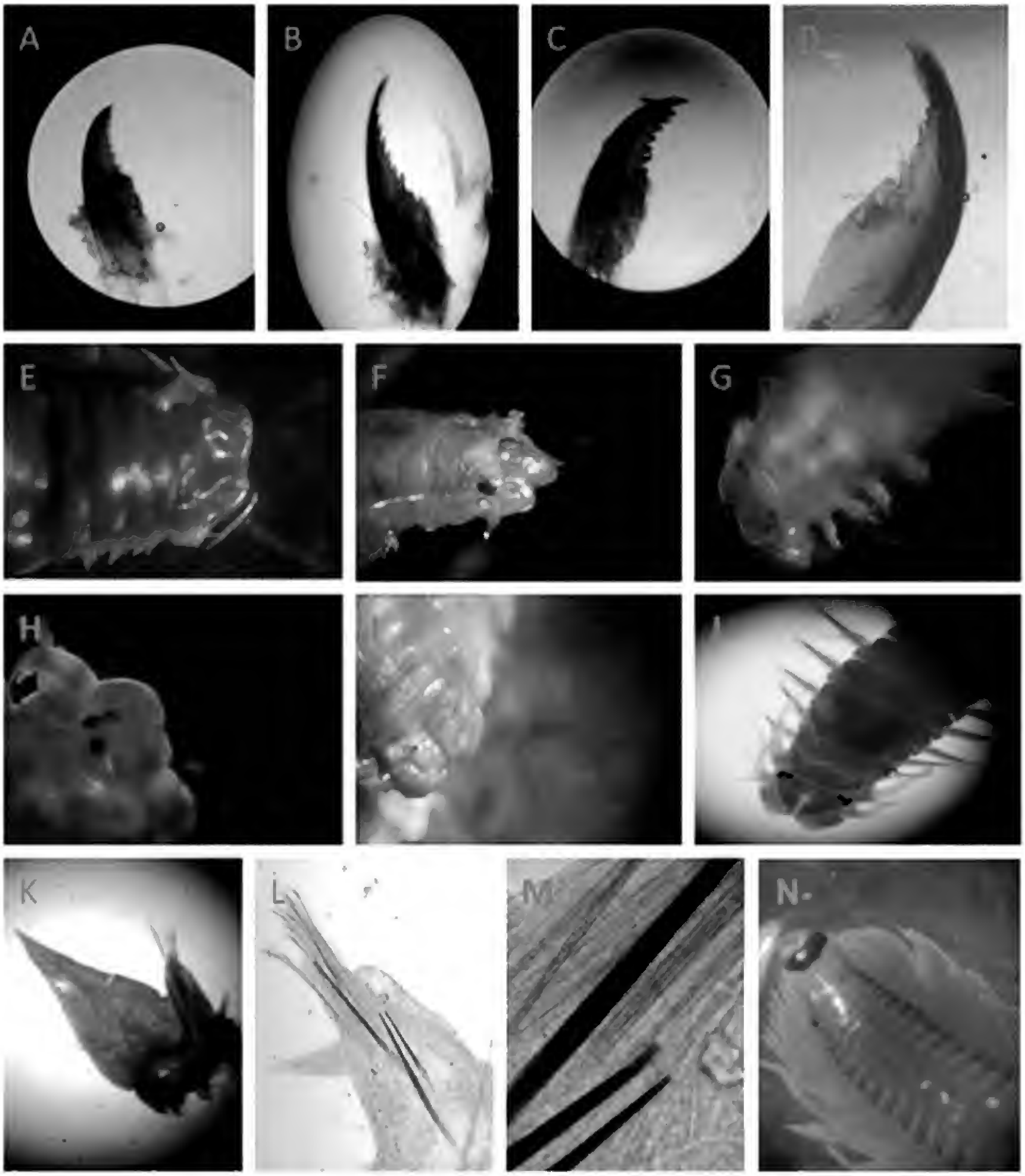

Figure 2. Selected morphological characters of the various *N. abiuma* species group morphotypes (M1-M6). A, M1, jaws with 11 teeth; B, M3, jaws with 10 teeth; C, M2, jaws with 9 teeth; D, M5 and M6 jaws with 8 teeth; E, M5, specimen with eyes absent; F, specimen with merged eyes; G-H, M5, specimens with three eyes; I, M5, specimen with faded eyes; J, M4, specimen with the longer tentacular cirri, indicative of species group 2 (see text); K, M4, specimen with relatively wider parapodium; L-M, M6, specimens showing three acicula; N, multi-incised pygidium.

bicanaliculata (GU362685) were downloaded from NCBI to allow for a wider range of interspecific comparisons, as well as phylogenetic analysis. The final alignment was devoid of inserted gaps, such that the sequences could be treated as pre-aligned. The genetic divergence comparisons between haplotypes are presented in Table 2. Genetic variation within the total dataset of '*N. abiuma'* specimens averaged 0.69% ± 0.21 and ranged between 0 (in several comparisons) and 0.99% (for specimens AQMM6, AQMM62-63 [haplotype H2] when compared against K5, K52-53 [haplotype H3]). As a reference, the average genetic variation between the '*N. abiuma'* haplotypes and *N. jaya,* and *P. bicanaliculata* was 1.42% ± 0.33, and 24.61% ± 0.19, respectively.

As a complement to the genetic distances, the resulting Neighbour-Joining (NJ) tree is presented in Fig. 3. The tree includes five main clades, four of which include representatives of the *N. abiuma* species group – specimens of *N. jaya* represent the remaining clade. These clades correspond to the haplotypes in the genetic variation analysis. This finding suggests some level of population structure and possibly incipient speciation within the specimens of the *N. abiuma* species complex treated in the present study. However, at the same time, the clades recovered in the NJ tree do not entirely reflect the morphotype separation. Specifically, the clade comprising specimens of haplotype H1 includes specimens displaying three different morphotypes (M1-M3; Fig. 3). The remaining clades each include only a single morphotype such that both morphology and molecules corroborate the separation of members of these clades.

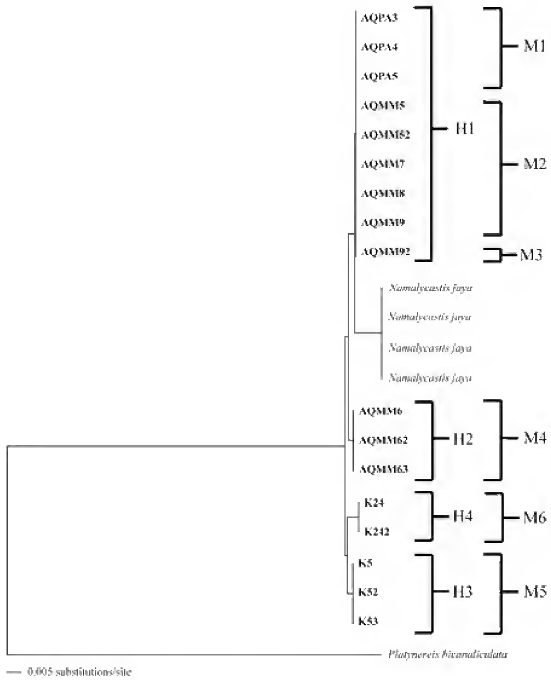

Figure 3. Neighbour joining tree derived from the COI dataset. Specimens pertaining to the *N. abiuma* species group are indicated in bold font, and morphotype (M1-M6) and haplotype (H1-H4) numbers, as referred to in text, are denoted by the brackets

Phylogeny

Nineteen out of the 509 aligned positions in the final COI dataset were parsimony informative. The heuristic search resulted in two equally parsimonious trees and these are presented in Figs. 4a and b, respectively. The resulting topologies are largely congruent with that of the NJ tree, as expected. Bootstrap support (BS) is low across the tree, most likely owing to the numerous identical haplotypes present in the dataset, in combination with the low number of parsimony informative characters. Notably, specimens of the *N. abiuma* species group do not form a monophyletic group, since specimens of *N. jaya* nest within the clade. In both of the most parsimonious trees, haplotype H2 (AQMM6, AQMM62 and AQMM63) is recovered as the sister group to the remaining taxa with bootstrap support (BS) of 61% (Figs. 4a and b). However, the trees disagree on the sister group of specimens pertaining to haplotype H1 (AQPA3-5, AQMM5, AQMM7-9, AQMM52 and AQMM92): in one of the trees, *N. jaya* is recovered as the sister group (BS <50%), whereas a clade containing haplotypes H3 and H4 (K24, K242, K5, K52 and K53) is recovered as the sister group of haplotype H1 in the remaining tree (BS 56%).

Discussion

In combination with the NJ and parsimony trees, the intraspecific versus interspecific variation within the dataset used here conclusively shows that the *N. abiuma* species group harbours more intraspecific diversity than previously noted. By and large, there is high congruence between the separation of morphotypes and haplotypes in the dataset. There is some evidence towards a separation, so far only at a population level, between haplotype H2 (specimens AQMM6, AQMM62 and AQMM63; also corresponding to morphotype M4) and the remaining haplotypes. This is supported both by morphology (specimens within morphotype M4 possess a wider parapodium and dorsal cirrus in middle and posterior chaetigers than other specimens), genetic distances (0.88% average distance when compared to remaining *N. abiuma* haplotypes) and phylogenetics (haplotype H2 constitutes a separate clade, as sister to the remaining specimens). Comparable patterns of congruence between morphology and molecules, when focusing on the separation of populations, were also recovered for haplotypes H3 (corresponding to morphotype M6) and H4 (corresponding to morphotype M7).

Both of the most parsimonious trees recover specimens of *N. jaya* nested within the major clade of the *N. abiuma* species group (albeit with negligible support). As a result, the detailed topology of the trees (Figs. 4a and 4b) further suggests the separation of haplotype H2 from the remaining taxa. This may indicate that haplotype H2 may be in a later stage of speciation than the remaining haplotypes, based on its phylogenetic

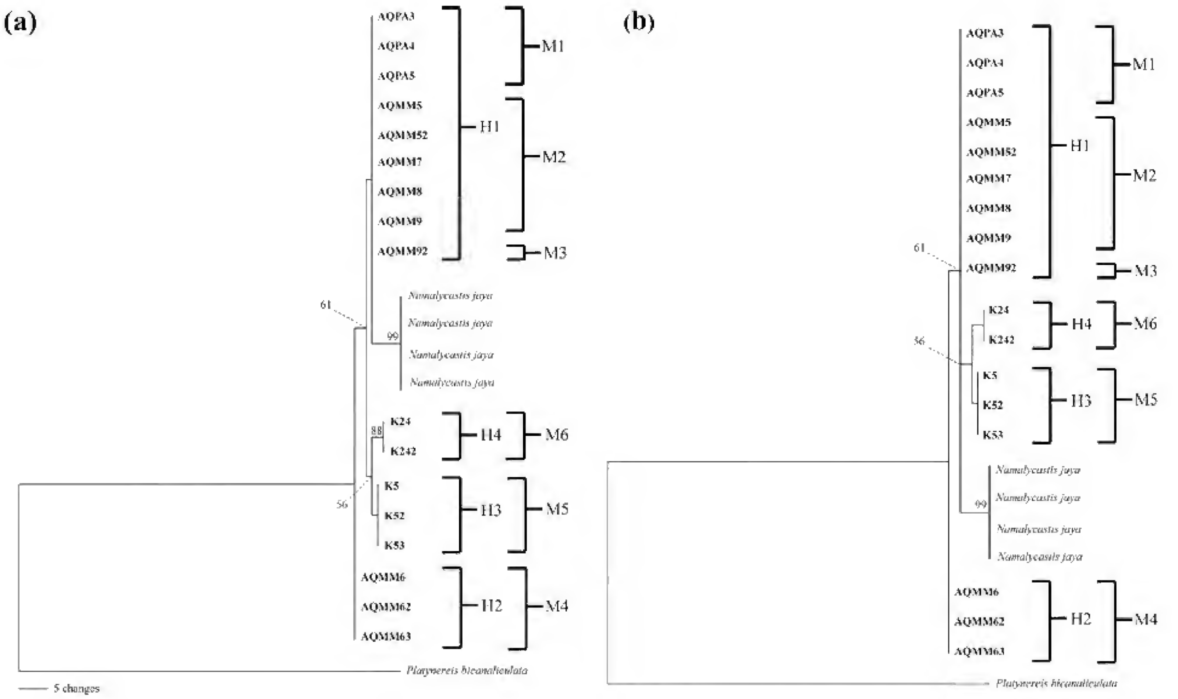

Figure 4. A-B shows the two equally parsimonious trees recovered from the phylogenetic analysis based on the COI dataset (length: 153, CI: 0.896, RI: 0.935). Specimens pertaining to the *N. abiuma* species group are indicated in bold font, and morphotype (M1-M6) and haplotype (H1-H4) numbers, as referred to in text, are denoted. Bootstrap support values above 50% are shown at each node. Branch lengths are drawn proportional to change.

position in the parsimony trees. However, the average COI genetic distance between haplotype H2 and the remaining specimens of the *N. abiuma* species group is lower than normal estimations of interspecific divergence (e.g., Hebert et al. 2003a, 2003b; Smith et al., 2005; Ratnasingham & Hebert, 2007). It thus seems premature to formally separate haplotype H2 from the remaining *N. abiuma* specimens, but our results suggest that these specimens may be in early stages of speciation.

The material included in the original description of *Namalycastis indica* was collected both from Calcutta and Cochin Backwater (Southern 1921) and specimens from the different locations differed slightly in their morphology (e.g. antennae shape, teeth count on jaw and size of tentacular cirri; Glasby 1999). The present study seems to include both of these variants as some specimens differed in their possession of shorter (Kadinamkulam and Cochin) or longer (Kayankulam) tentacular cirri. However, a number of globally distributed *Namalyastis* species have previously been incorrectly described as distinct separate species, and several of these were later assigned to the *N. abiuma* species group (although not formally synonymised; see Introduction) by Glasby (1999). These include *Lycastis meraukensis* (Horst, 1918; Fauvel, 1932), *Lycastis indica* (Horst, 1924; Fauvel, 1932; Aziz, 1938; Ghosh, 1963), *Namalycastis* cf. *abiuma* (Hutchings & Glasby, 1985), *Lycastis nipae* (Pflugfelder, 1933), *Lycastis vivax* (Pflugfelder, 1933), *Lycastis senegalensis* (Monro, 1939), *Lycastris* [sic] *indica* (Day, 1951), *Namalycastis rigida* (Pillai, 1965) and *Namalycastis meraukensis zeylanica* (Silva, 1961) (see Glasby (1999) for a full account of synonyms). Therefore, it would be premature to conclude that some specimens of the present study indeed represent both variants found by Southern (1921).

One of the strangest findings of the present study is the occurrence of three eyes (in one side) and three acicula (10th chaetiger) in morphotype M2. This is the first record of a *Namalycastis* species possessing such a set of characteristics. However, a more rigorous and taxon-inclusive morphological assessment is needed prior to drawing any conclusions. For example, it is still possible that the polluted environment of the Kayamkulam collection area is the cause of this oddity. This is particularly plausible, seeing as the genus already presents several adaptations of the eyes to low-salinity or semi-terrestrial habitats (Sadasivan Tampi 1949, Storch & Welsch 1972).

In conclusion, the *N. abiuma* species group does seem to possess a higher degree of diversity than currently reflected in the taxonomy, as was suggested by Glasby (1999). Because of the somewhat confusing morphology of these species, it is important that future studies also include molecular information.

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Morphology, feeding and behaviour of British *Magelona* (Annelida: Magelonidae), with discussions on the form and function of abdominal lateral pouches

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Abstract

Mortimer, K. and Mackie, A.S.Y. 2014. Morphology, feeding and behaviour of British *Magelona* (Annelida: Magelonidae), with discussions on the form and function of abdominal lateral pouches. *Memoirs of Museum Victoria* 71: 177–201.

Observations were made on *Magelona johnstoni* Fiege, Licher & Mackie, 2000 and *Magelona mirabilis* (Johnston, 1865) maintained in a laboratory aquarium. Burrowing, feeding, palp regeneration, lateral pouch function, and behaviour were studied. The two morphologically similar (and co-occurring) species exhibited different behaviours and feeding strategies. Individuals of *M. johnstoni* were seen to undertake lateral sinuous movements of the thorax, both within and outside the burrow. These movements often occurred simultaneously in several animals, and on occasion, semi- emergent pairs also made direct thoracic contact. This behaviour generally took place between April and July and was likely associated with reproduction; published works suggest spawning may take place between May and August. The morphology and function of abdominal lateral pouches was investigated through SEM images, experimental observation, and consideration of literature accounts.

Keywords

live observation, polychaete, burrowing, feeding, functional biology, lateral pouch, reproduction, palp regeneration, *Magelona johnstoni*, *Magelona mirabilis*

Introduction

The Magelonidae is a small family of polychaete worms, with around 70 species described worldwide. Most species are included in the genus *Magelona* F. Müller, 1858; however, two further genera have been described, *Meredithia* Hernández-Alcántara & Solís-Weiss, 2000 and *Octomagelona* Aguirrezabalaga, Ceberio & Fiege, 2001.

Magelonids are common in sands and muds, both intertidally and subtidally; most species occur in shallow waters (<100 m). They have a characteristic flattened prostomium, which gives rise to the group's common name, the shovelhead worms. Two long papillated palps arise ventral to the prostomium, one either side of the mouth. Magelonid bodies are divided into two regions: a thorax of eight or nine segments, and an abdomen of many segments. Very little information about the biology, anatomy and behaviour of these animals exists; most existing knowledge comes from the works of McIntosh (1877, 1878, 1879, 1911, 1915, 1916) and Jones (1968). Filippova et al. (2005) investigated the musculature of *Magelona* cf. *mirabilis* by phalloidin labelling and confocal laser scanning microscopy (cLSM), while Dales (1962, 1977) and Orrhage (1973) provided details on the magelonid buccal region and proboscis. Brasil (2003) examined the phylogeny of the Magelonidae based on external morphological features. Relatively little is known about the reproductive biology of the group (Rouse, 2001; Blake, 2006), and most knowledge of magelonid larval development comes from Wilson (1982).

Jones (1968) made observations on an unnamed species of *Magelona* collected near Woods Hole, stating that it was "more closely related to, but not identical with, the species referred to as *M. papillicornis* F. Müller by McIntosh (1877, 1878, 1879, 1911, and 1915) and other European workers". This species was not subsequently formally described. Few other studies of living magelonids exist. The present study aims to increase our knowledge of several British magelonid species: primarily *Magelona johnstoni* Fiege, Licher & Mackie, 2000 and *Magelona mirabilis* (Johnston, 1865). Most European records of *Magelona papillicornis* Müller, 1858 (a Brazilian species) have been attributed to these two species, after the works of Jones (1977) and Fiege et al. (2000), and therefore the *Magelona* sp. of Jones (1968) is likely to share similarities with them and have great relevance to our study.

One of the main diagnostic features within the Magelonidae is the presence or absence of lateral abdominal pouches. Fiege et al. (2000) described two types of lateral pouch present within the family:

- ∑-shaped pouches occur on the anterior abdomen and are generally paired on either side of the body. They are bounded, dorsally and ventrally, by a cuticular flap, containing a convoluted membrane, and open anteriorly.
- C-shaped pouches open posteriorly, occurring on median and posterior abdominal chaetigers. They are simple, pocket-like, and appear C-shaped when viewed in cross-section. They may be unpaired, alternating from one side of the body to the other, on alternate segments, or paired on consecutive segments.

Unfortunately, mention of magelonid pouches within species descriptions has been somewhat vague. Although Uebelacker and Jones (1984) stated: "In some species, lateral pouches occur between the parapodia of two consecutive anterior abdominal parapodia, or anterior to the parapodia of some or all segments farther back", it was not until the work of Fiege et al. (2000) that different pouch morphologies in magelonids were described more fully. Many species descriptions prior to this noted only presence or absence, made no mention whatsoever, or incorrectly reported absence of pouches. The last two situations have been particularly true for species where the first pouch appears in the posterior region of the animal (e.g. *Magelona filiformis* Wilson, 1959 or *Magelona dakini* Jones, 1978—appearing after the 100th chaetiger, see appendix), or for species described from anterior fragments only. Reporting of anteriorly opening pouches was generally more reliable due to their conspicuous nature in comparison with posteriorly opening pouches. Patterns in pouch location distribution are reported more widely nowadays, and, more recently, additional pouch morphologies have been recognised: e.g. medial slits of posteriorly opening pouches (Mortimer, 2010: 22).

The function of these lateral pouches is unknown. Fiege et al. (2000) observed no independent motion of pouches for *M. johnstoni*, only contraction and expansion associated with movement. However, based on a personal communication from Leslie Harris, they reported irregular pouch contractions for *Magelona sacculata* Hartman, 1961, first on the dorsal side and then on the ventral side. Jones (1968) stated that the function of pouches in *Magelona* species would not seem to be related to reproduction, since they are present in males, females and juveniles, and neither Jones (1978) nor McIntosh (1911) found any communication from the interior of the animal through to the pouches.

To gain a better understanding of the biology of magelonids and to investigate the possible function of lateral pouches, detailed observation of live material was made in the laboratory. Additional study on pouch morphology was made using Scanning Electron Microscopy (SEM).

Materials and methods

Animal collection

Animals were collected over a 5-month period (November 2012 – April 2013) from three separate beaches (Rhossili Beach and Oxwich Bay, South Wales; and Berwick-upon-Tweed, Northumberland, north-east England) at low water (tide height of 0.9 m or less). Animals were gently removed from the sediment by hand using wash bottles and pliable forceps, after digging. Three species were collected: *M. johnstoni* (fig. 1), *M. mirabilis* (fig. 2) and *M. filiformis*. Animals were placed in small containers with seawater (a few individuals per container to prevent entanglement) and kept cool in iceboxes during transportation. The samples were processed within the laboratory as soon as possible after collection.

Tank and cooling system

An aquarium tank (45 × 20 × 20 cm), holding ~11 L of artificial seawater, was chilled by means of a closed water system (fig. 3A). Water was circulated by an AquaManta EFX 200 External Filter passed through a D-D DC300 aquarium cooler and into a coiled tube running along the bottom of the tank (kept in place between two layers of plastic mesh), before returning to the filter for circulation. A plastic shelf on top of the coiled pipe provided a flat surface on which smaller observation tanks were placed. Pipes between the filter, cooler and tank were lagged to prevent condensation and help maintain the experimental temperature. The water in the closed system was kept at a constant temperature (within ±1.5°C), with the aquarium water ~3–5°C higher (depending on the ambient temperature of the laboratory). The aquarium temperature was initially set to 6–7°C but was increased in parallel with sea surface temperatures for Northumberland as observations progressed (i.e. ranging from ~6°C in winter to ~14°C in summer). A standard aquarium pump and large air stone was employed to aerate the water and create a current within the tank.

Capillary tube observations

Two sizes of non-heparinised capillary/melting point tubes (80 mm in length, closed ends removed with a hand-held rotary tool), with internal diameters of 0.80 mm and 1.1 mm were used. In general, smaller diameter tubes were used for *M. johnstoni* and larger diameter tubes for *M. mirabilis*. It was important to select the right diameter tube for each individual; if too large, they were unable to crawl inside or would not remain inside. If the tubes were a good fit, then worms would quickly move up the inside, stopping ~3 cm from the end before looping their palps out (fig. 3B). Bubbles were removed before the addition of animals by placing a plastic pipette (cut to the right diameter) on the end of the capillary tube and sucking water through.

In initial experiments, animals were removed from the sediment and the prostomium placed gently into the end of the tube using forceps. The worms were then 'encouraged' to crawl in by gently tapping the posterior. However, in later experiments animals were left in the sediment and the end of a capillary tube placed near their prostomia. In most cases, they would crawl into the tubes after a short period of time, decreasing handling and the likelihood of damage during sediment removal. The capillary

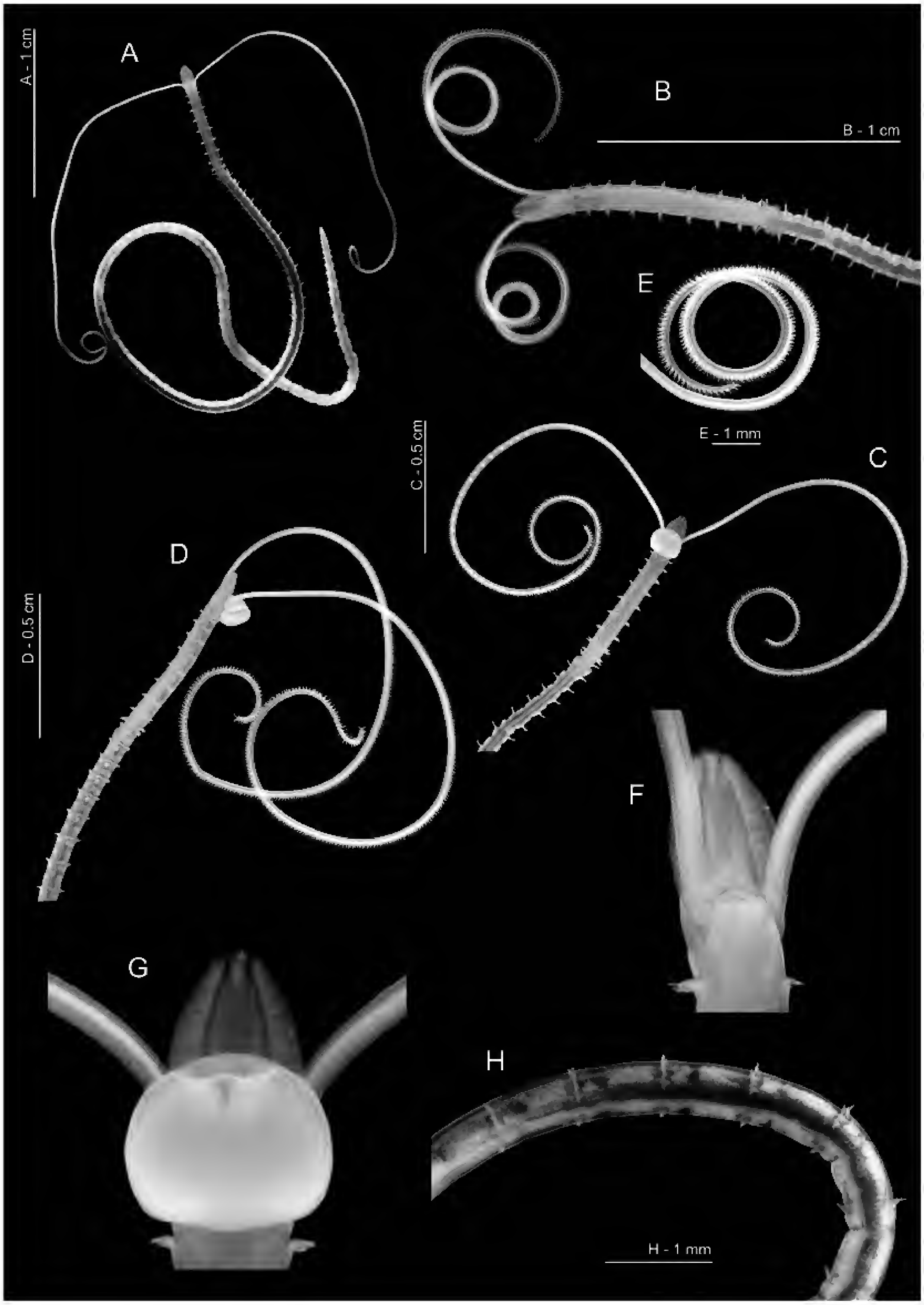

Figure 1. *Magelona johnstoni* Berwick-upon-Tweed (A, C, D, G: NMW.Z. 2013.037.0018; B: NMW.Z. 2013.037.0001; E, F: NMW.Z. 2013.037.0017; H: NMW.Z. 2013.037.0015): A, whole animal; B, anterior (dorsal view); C, anterior (ventral view); D, anterior (lateral view); E, palp; F, prostomium (ventral view, showing mouth); G, prostomium (ventral view, 'proboscis' everted); H, posterior section of female showing eggs. All $MgCl_2$-relaxed. Photos: A.S.Y. Mackie.

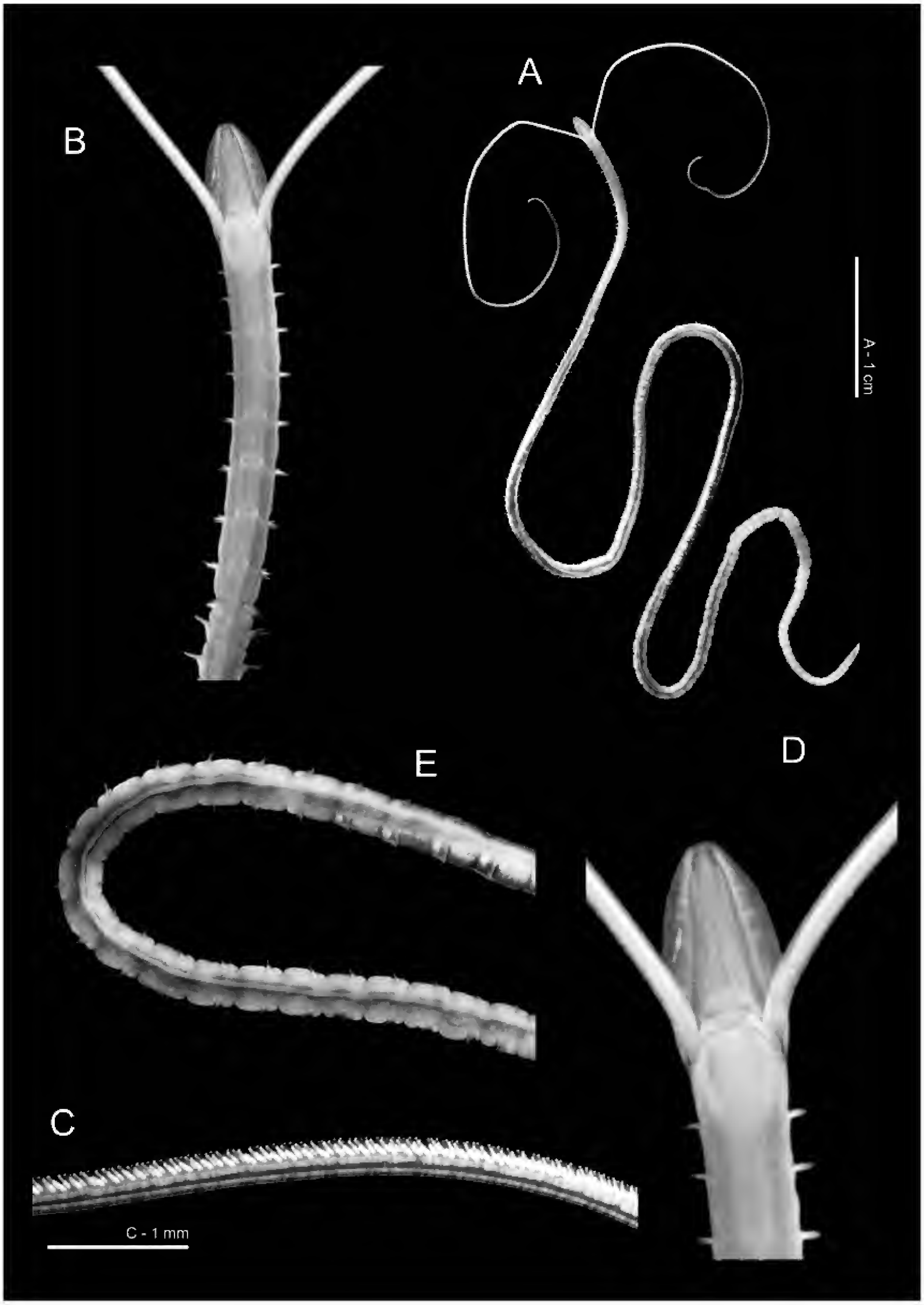

Figure 2. *Magelona mirabilis* Berwick-upon-Tweed (NMW.Z. 2013.037.0020): A, whole animal; B, anterior (ventral view); C, palp; D, prostomium (ventral view, showing mouth); E, posterior. All $MgCl_2$-relaxed. Photos: A.S.Y. Mackie.

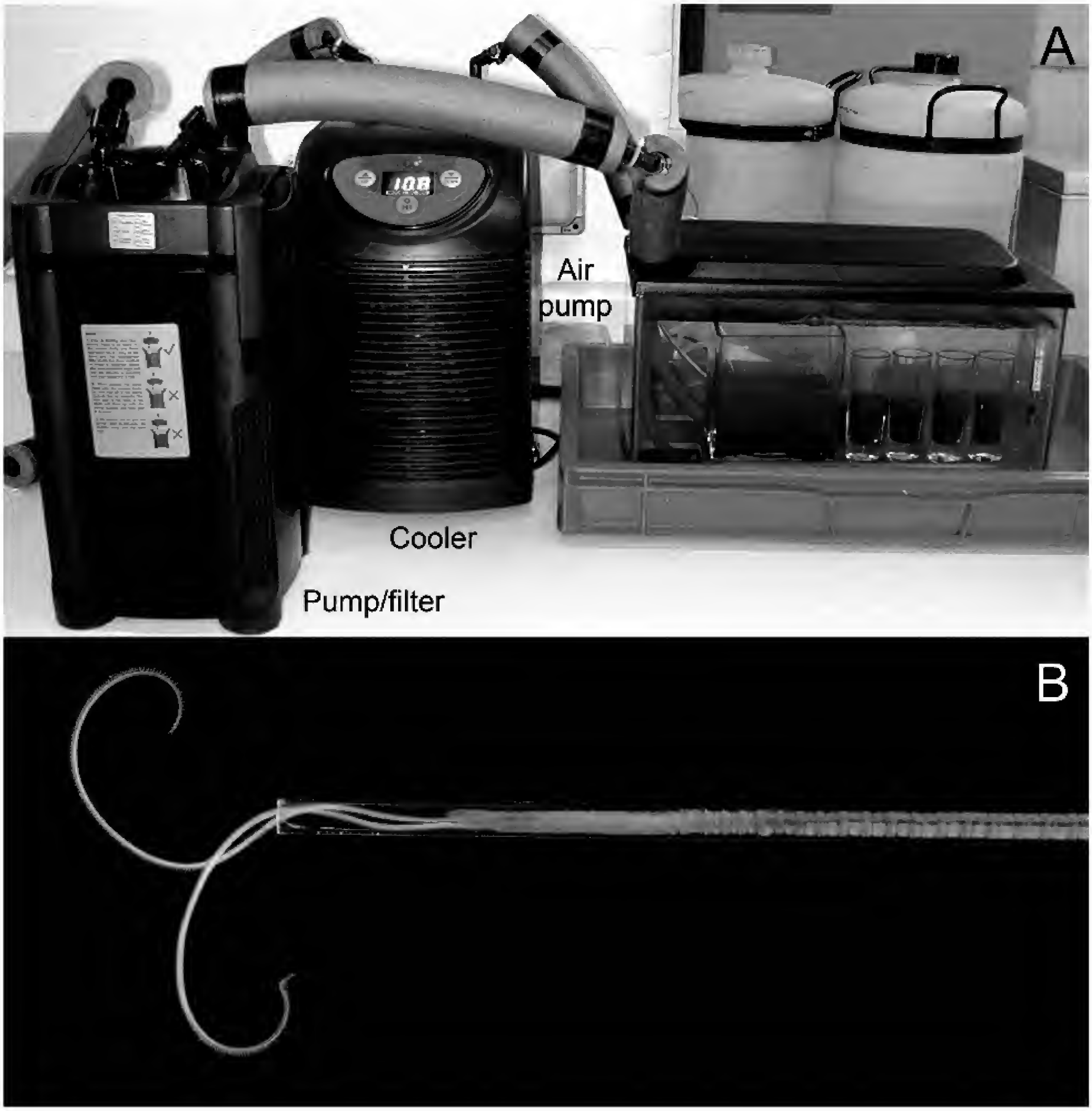

Figure 3. Experimental set-up: A, aquarium tank and cooling system; B, *Magelona johnstoni* Berwick-upon-Tweed (NMW.Z. 2013.037.0001): live animal in capillary tube. Photo: A.S.Y. Mackie.

tubes were then placed in small observation tanks within the main aquarium, some capillary tubes on the bottom of the tank and others held upright using a small plastic table-shaped holder. Capillary tubes were removed from the tank at intervals and viewed under a Leica MZ9.5 zoom microscope.

Additional observations within capillary tubes primed with a weak carmine or food colouring solution were carried out under a microscope. Carborundum powder was also tested (particle size ~36 μm) but proved to be too coarse and dense.

In situ *laboratory experiments*

Sediment from the sampling site was sieved through a 0.5-mm sieve to remove macrofauna, while trying to retain the sediment characteristic of the sample. This was placed into a

small glass tank (internally 11.3 × 11.3 × 11.5 cm; volume ca. 1470 cm^3) and allowed to settle before adding magelonids. Further sediment was placed on top and allowed to settle in a fridge before placing into the aquarium. In earlier trials, worms were placed directly onto the surface of the sediment, but many were unable to penetrate the surface so this second technique was adopted. Experiments were carried out in both still and flowing water to observe any potential differences in behaviour. The sediment level within the observation tanks was increased (to 5.5 cm deep, ca. 700 cm^3 volume) during observations between April and June 2013, both to increase water flow across the sediment and allow a greater depth for burrowing.

Food was added to the tank at the sediment surface or around capillary tubes every 4–7 days, using plastic pipettes. Several food options were utilised: frozen marine invertebrate aquarium food (Dutch Select foods—food for invertebrates, marine) and SeAquariums Invertfood liquid diet (made up of plankton and other essential marine nutrients). Food was mixed with flocculent material collected from the surface of the sediment during sampling, enabling it to sink towards the sediment surface.

Animals were observed for seven months (April–October) during daylight hours; no observations were made at night. All experiments were filmed with a miniDV camcorder, and the resulting footage was observed both at full speed and in slow motion (10–50% slower). Separate glass tanks within the main aquarium were utilised, each containing only one of the species (*M. mirabilis* or *M. johnstoni*), allowing direct comparison of their behaviour. A further two smaller tanks were used, one containing animals that had lost both palps upon collection and one containing those that had lost only one. Palp regeneration was then followed over a period of 40 days for *M. johnstoni*. Animals were observed using a low-powered zoom microscope (×15–30) held horizontally towards the tank. Food colouring and carmine particles were added to the surface waters of small isolated tanks holding individual animals, in order to observe water flow.

Scanning electron microscopy (SEM)

Additional animals collected for SEM were fixed in ca. 6–8% formaldehyde or glutaraldehyde in seawater. Specimens were subsequently washed with fresh water, and transferred in an alcohol series through to 100% ethanol for critical point drying. They were then Sputter coated before imaging using a Jeol Neoscope JCM-5000 SEM. Specimens have been deposited in the National Museum of Wales (NMW), Cardiff.

Current knowledge of pouches in magelonids

All magelonid species descriptions and re-descriptions were examined for details of pouch presence/absence, pouch type (anteriorly or posteriorly opening), configuration (paired or unpaired), pattern (on alternating segments or consecutive segments) and the segment at which they first occur. The resulting information was then compiled to identify groups of species.

Observations and Discussion

Species presence and abundance

Each of the selected sampling sites varied in terms of sediment characteristics and consequently differed in the species present and their relative abundances. *Magelona johnstoni* was most abundant in the silty fine sands of Berwick-upon-Tweed, while *M. filiformis* dominated in the fine sands of Oxwich Bay. *Magelona mirabilis* was collected in low numbers at all sites, but *M. johnstoni* was absent from collections made at Oxwich Bay. *Magelona* were difficult to consistently collect on the Rhossili Bay shore due to its susceptibility to onshore winds and waves, though all three species were known to occur there, and sublittorally (Mackie et al. 2006). Hence, *M. johnstoni* and *M. mirabilis* were conveniently sourced from Berwick-upon-Tweed, and *M. filiformis* was collected at Oxwich Bay. Unfortunately all material of *M. filiformis* was small and delicate, and mortality occurred within several days. No observational data was obtained for this species.

Of the two remaining British species, *Magelona alleni* Wilson, 1958 was only recorded once during preliminary sampling at Mumbles Bay, Swansea (March 2012) and, from previous collecting (1998–2012), was known to be infrequent at Berwick-upon-Tweed. *Magelona minuta* Eliason, 1962 is an offshore muddy sediment species and was not encountered on any of the shores.

As previously mentioned, European records of the Brazilian *M. papillicornis* actually relate to *M. mirabilis* or *M. johnstoni,* or both. The same situation holds for any pre-2000 account of *M. mirabilis* (see Fiege et al. 2000). In the following text, an asterisk identifies these erroneous or suspect citations as *M. papillicornis** or *M. mirabilis**.

Burrowing

Burrowing observations for *M. johnstoni* essentially match those described by McIntosh (1878; 1911) for *M. papillicornis** and Jones (1968) for *Magelona* sp. When burrowing, *M. johnstoni* moved its prostomium laterally from side to side, loosening the sediment in front and aiding movement forward. The everted 'proboscis' (see Mortimer et al., 2012 regarding terminology) was used as an anchor, allowing the body to be pulled towards the head. The 'proboscis' was then retracted, the prostomium moved forward and the process repeated. Jones (1968) felt that eversion of the 'proboscis' occurred primarily due to the hydrostatic pressure of the blood, but to a lesser extent via that of the coelomic fluid. The 'proboscis' is therefore totally essential for burrowing, and if compromised, would likely be fatal for the worm. This was recognised by McIntosh (1915), who suggested that the group's preference for fine sands may help avoid sharp fragments of coarse gravel and sand that might damage their proboscides.

Jones (1968) postulated that the hollow cylindroid dorsal muscular ridges of the magelonid prostomium, which are provided with longitudinal muscles, were presumably fluid-filled and likely to provide rigidity during burrowing. During burrowing, the palps trailed behind the body, but once the worm was near the sediment surface, the palps looped out from underneath the body towards the opening. Both *M. johnstoni*

and *M. mirabilis* were observed to burrow directly to the surface of the sediment and then withdraw into the burrow. Alternatively, they stopped before the surface and moved their palps through the sediment to the water column. Palp length in living animals was extremely long (figs 1A–E; 2A), and the worms could stay well within the burrow with only the last distal sixth of the palps projecting into the water column (fig. 4A).

In the laboratory, *M. johnstoni* generally burrowed horizontally within the sediment. This was consistent with field observations, collected animals being found with the same orientation within the sediment at Berwick-upon-Tweed. To commence feeding, worms then burrowed upwards from their horizontal position towards the surface, thus, creating an arched or diagonally shaped burrow opening out into the water column (fig. 4A). Some variation in burrow shape was observed, although no U-shaped burrows were seen. McMahon and Jones (1967) and Jones (1968) suggested that *Magelona* sp. constructed vertical burrows. The latter author described animals burrowing directly downwards once initially placed into the observation chamber, then after reaching the bottom, they burrowed up to the surface. Although for our *M. johnstoni*, burrow shape was straighter in deeper sediment, strictly vertical burrows were not usually seen.

Differences in observed behaviours could be due to the contrasting experimental chambers and the methodology of both studies. The chamber used by McMahon and Jones (1967) was constructed from a U-shaped rubber tube clamped between two pieces of glass plate, which were no more than 0.7–1.0 cm apart (McMahon pers. comm.), and worms were introduced to the sediment surface. As stated above, *M. johnstoni* struggled to penetrate the sediment when placed directly onto the surface, therefore additional sediment was allowed to settle upon the worms after their placement into the cube-shaped tank. This may have affected the direction of initial travel; however, the much broader tank would not have constrained the direction of burrowing. The sediment volume in the observation tank used here was ~700 cm^3, allowing ample space for movement in any direction, unlike the narrow tank of McMahon and Jones (1967) and Jones (1968). Nevertheless, once settled, *M. johnstoni* often burrowed against the glass of the observation tank, allowing them to be fully observed. Whether this was fortuitous, the worms were simply burrowing until they reached the glass, or it was due to an attraction to food accumulated against the tank sides, was not determined. The undescribed *Magelona* from Woods Hole was shown to have U-shaped burrows (McMahon, pers. comm.), with both ends at the surface. This warrants further investigation, particularly between species, and may depend on an ability to burrow backwards as well as forwards within a burrow.

During feeding and resting within the burrow, the bodies of both *M. mirabilis* and *M. johnstoni* were greatly stretched, their abdomens somewhat narrower than the thorax. In this region, only the lamellar tips were in contact with the sides of the burrow (fig. 6). If disturbed, contraction of their bodies enabled both species to withdraw quickly into their burrows. However, neither seemed able to actively burrow backwards. To change direction, bodies were retracted, bringing the prostomia under the sediment surface and new burrows were formed in other directions. Before initiating a new burrow, individuals blocked the ends of their old burrows by shaking their prostomia laterally while everting their proboscides.

Burrows were observed to be temporary and worms moved around, periodically after several hours or days, making new burrows. Fauchald (1983) observed similar behaviour for *M. sacculata* living in sandy substrates off southern and central California. This species appeared to move through the sediment on a more or less continual basis. Movement to a new burrow may be initiated by the need to locate further food sources. However, *M. mirabilis* moved around less frequently than *M. johnstoni* and was generally much less active. Permanency of burrows may well be species-specific; species such as *M. alleni* and *M. cincta* Ehlers, 1908 build recognisable tubes (Mortimer and Mackie, 2009; Mortimer et al., 2012).

Burrows appeared to be maintained by mucus, something noted previously by Jones (1968) for *Magelona* sp. and Wilson (1982) for metamorphosing/metamorphosed *M. filiformis*, *M. alleni* and *M. mirabilis**. Wilson further noted that some larvae used a band of mucus for adhesion in the approximate region of the ninth adult chaetiger. Interestingly, we have seen adults of *M. alleni*, separated from their usual red-purple papery tube (during the sieving of grab samples) quickly produce a loose mucus-bound sand tube (pers. obs.). Mucus secretions for *M. johnstoni* appeared much greater than those for *M. mirabilis* in the laboratory.

The longevity of magelonids kept in glass capillary tubes was much reduced in comparison with those living unconstricted in the aquarium sediment. Animals kept in capillary tubes within the aerated tank lasted about 4 days only. This was in marked contrast to the success others have had in maintaining various Spionida for long periods of time (over 4 years) in capillary tubes within petri dishes (Williams, 2002; Dualan and Williams, 2011). This dependence on sediment was recorded by McIntosh (1911), "sand is very necessary for the existence of this form, for though the animals survive a considerable period in captivity in vessels filled with pure sea-water, they thrive much longer amongst fine sand, with a few inches of water over it". In our study, one individual kept in a capillary tube held upright in sediment, survived for over 8 weeks.

Buccal region

The buccal region of *M. johnstoni* has three lips, one larger triangular lip above two smaller lateral lips (fig. 1F, fig. 5A). These were seen to expand and separate, revealing a triangular-shaped mouth at their centre (fig. 4F). Animals displayed a 'gulping' action, apparently sucking in water on opening the mouth. The surface of the top lip and the area just above in *M. johnstoni* is speckled (fig. 1F).

Our observations agreed with those of the mouth of *M. papillicornis** as described by McIntosh (1911), "a somewhat triangular or T-shaped slit surrounded by lips of mucous membrane, and situated between or very slightly in front of the bases of the tentacles. The anterior lip is sinuous but complete, while inferiorly there is a wide fissure (bounded laterally by prominent margins), which runs a considerable distance backwards. The lips are very mobile and in life frequently expand to gulp water."

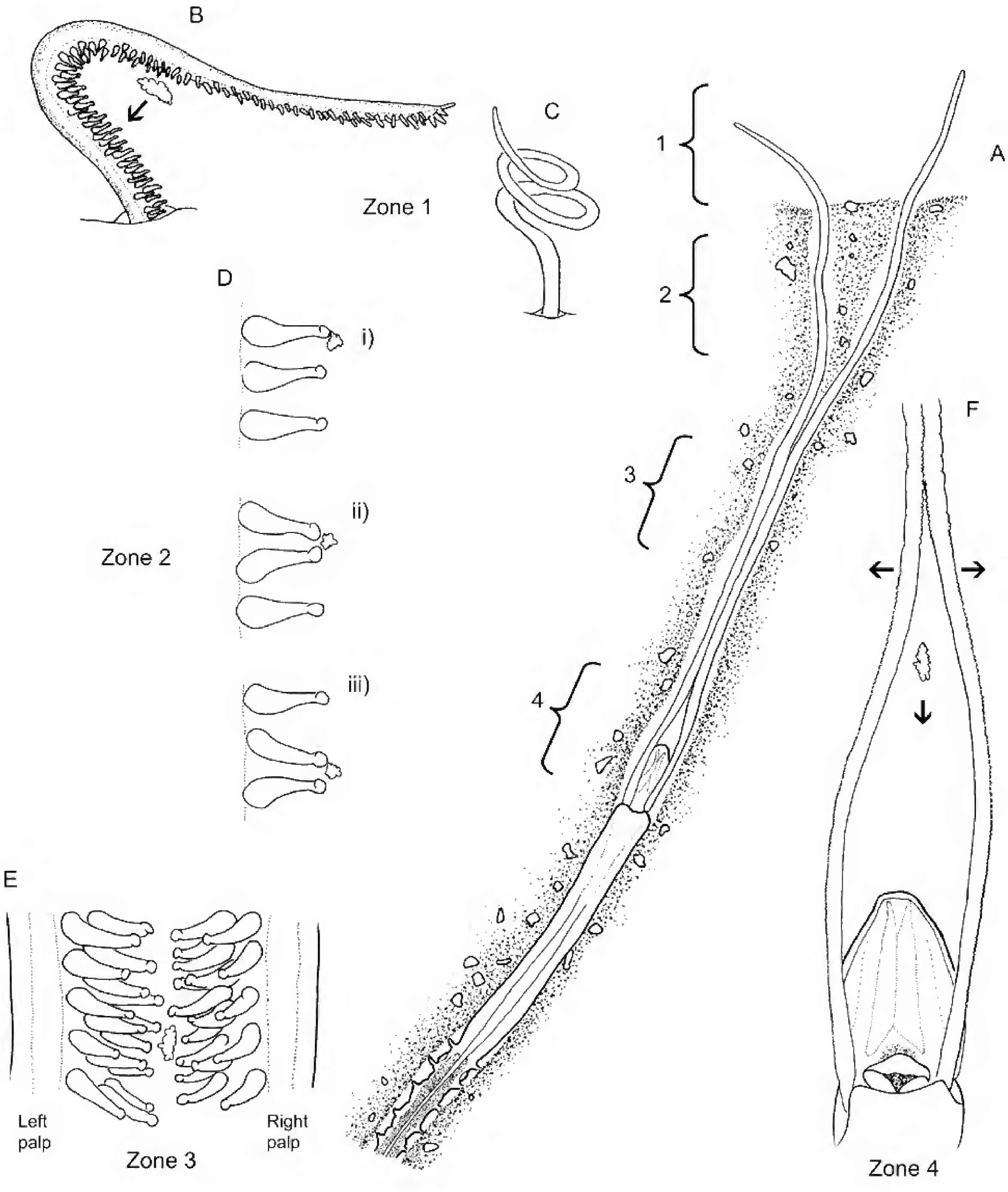

Figure 4. Feeding in *Magelona johnstoni*: A, feeding position within the burrow (ventral view), indicating four zones where different methods are utilised to move food particles along the palp; B, looping of the palp at the surface (zone 1), in order to pass food particles along the palp (lateral view); C, similar process to that shown in B but utilising coiling of the palp (lateral view, papillae omitted for clarity); D, sequence showing the process of passing food particles from papillae to papillae along palp in zone 2; E, food particles being passed between papillae of both palps in zone 3; F, region where food particles are dropped towards mouth (ventral view) in zone 4.

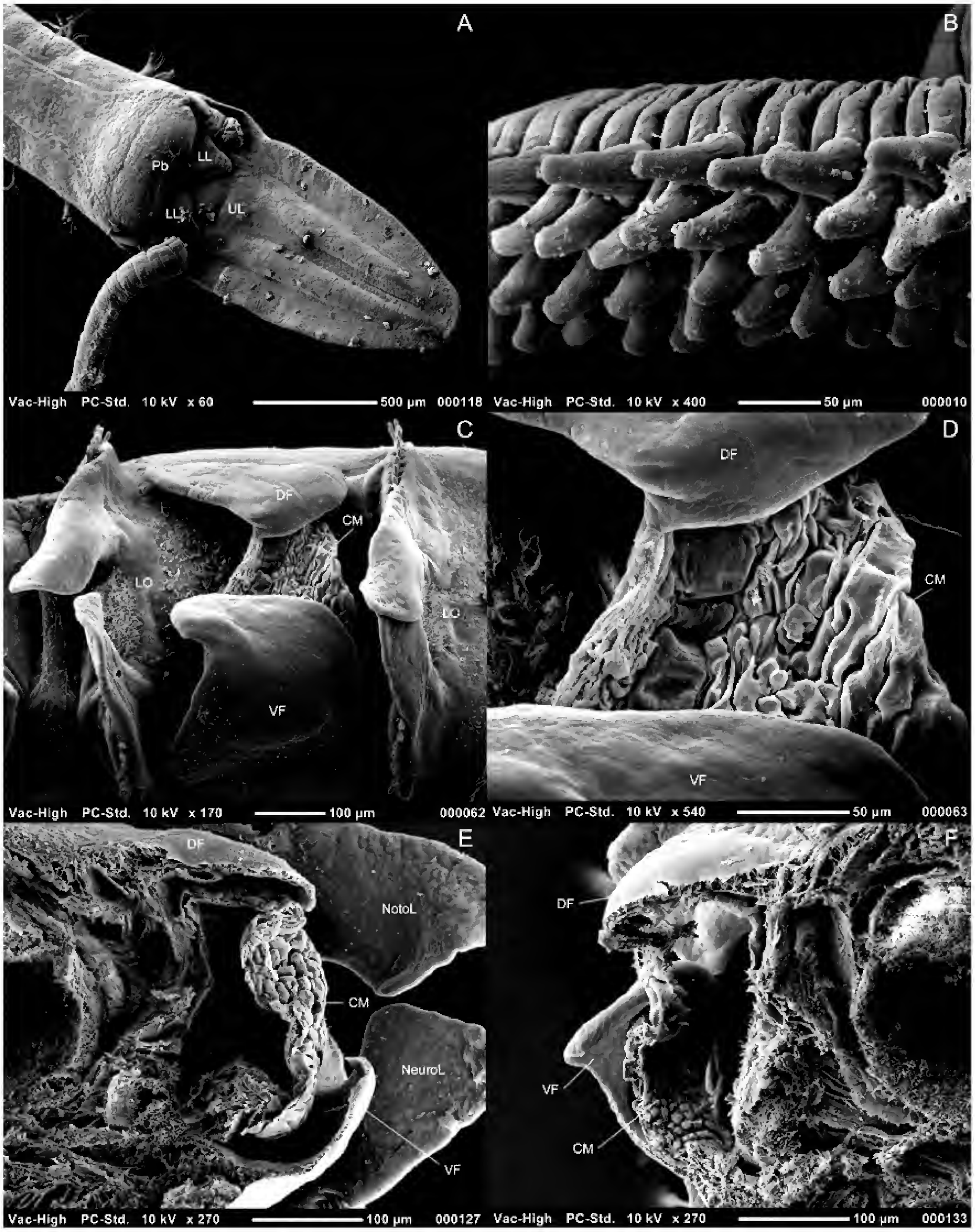

Figure 5. *Magelona johnstoni* Berwick-upon-Tweed: A, prostomium and first two chaetigers (ventral view), showing mouth surrounded by one upper (UL) and two lower lips (LL), and the proboscis (Pb, not everted) (NMW.Z.1999.021.0020a); B, papillae of mid-palp region (NMW.Z.2013.037.0008c); C, left-hand anteriorly opening pouch located between chaetigers 10 and 11 (lateral view, DF = dorsal flap, VF = ventral flap, LO = lateral organ, CM = convoluted membrane) (NMW.Z.2013.037.0011b); D, close-up view of convoluted membrane; E, transverse section through the body and anteriorly opening pouch situated between chaetigers 10 and 11 (posterior half of pouch and parapodia of chaetiger 11) (NotoL = notopodial lamellae, NeuroL = neuropodial lamellae) (NMW.Z.2013.037.0010c); F, anterior half of same pouch (NMW.Z.2013.037.0010b). Photos: K. Mortimer.

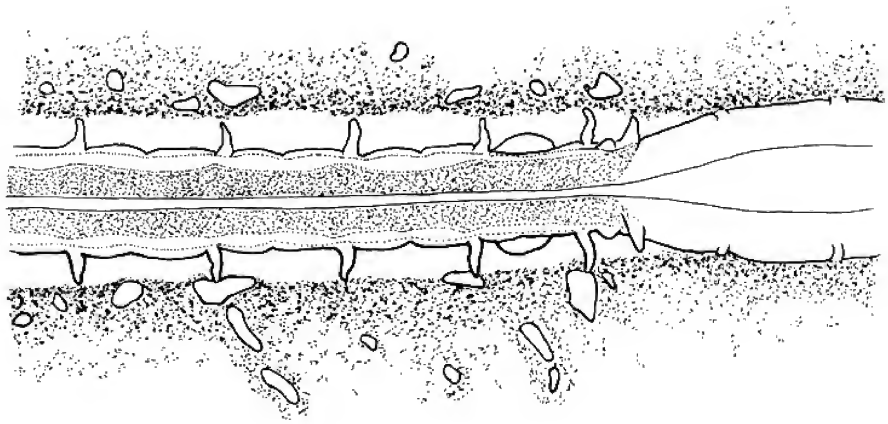

Figure 6. *In situ* picture of the posterior thorax and anterior abdomen of living *Magelona johnstoni* (chaetigers 7–14, dorsal view).

The buccal region of *M. mirabilis* (fig. 2D) functioned differently. When feeding, a more extendable extension ('buccal tube') of the alimentary canal appeared to be present, and 'gulping' was not observed. However, feeding observations in this species were relatively infrequent and further investigations are warranted. A 'buccal tube' was previously reported for *Magelona* cf. *agoensis* Kitamori, 1967 (Mortimer et al., 2012: Fig. 4), and the length to which it can be protruded seems to be species specific.

Palps and feeding

Magelona johnstoni and *M. mirabilis* both remained well within their burrows during feeding, projecting only the distal sixth of their palps into the water column (fig. 4A). This is in contrast to records by Jones (1968), who stated that once *Magelona* sp. worms had reached the surface, they would withdraw only several millimetres and extend their palps as much as 15 to 20 mm into the water column. This was equivalent to one quarter of the worm's length (McMahon and Jones, 1967). In general, our animals only extended their palps up to 4 mm above the sediment surface during feeding. Greater extensions of the palps above the sediment were observed in *M. johnstoni* in still water conditions when the air stone was turned off, and in these conditions, they would keep the palps stiff and displayed in a V-shape. McIntosh (1877) stated that they could extend to two inches (5 cm) in *M. papillicornis**, "with the capability of even greater elongation". His figure (McIntosh 1878: pl. XXXVIII, fig. 1) showed three-quarters of the palp emerging. McIntosh (1911) later noted that *Magelona* "projects its extremely elongated tentacles through the aperture of its tube into the surrounding water, in which they are jerked to and fro, stand stiffly out, or are gracefully curved and moved in a serpentine manner here and there over the sand".

In our studies, the palps of *M. johnstoni* showed one of three arrangements when exposed in the water column: stiff and V-shaped with tips pointing upwards, arched with tips on the surface on the sediment, or gently waving. When a current was flowing, *M. johnstoni* tended to wave its palps gently and laterally within the water column. The individual palps of each animal frequently exited the sediment via different holes, often in different directions. These separate holes were connected ~5 mm below the sediment surface (fig. 4A). In capillary tube experiments, palps were extended further outside to sense the environment (fig. 3B), but were rapidly withdrawn in response to any vibrational stimuli.

The addition of food particles to the observation tank caused an instant reaction in *M. johnstoni*; palps were waved more rapidly within the water column and across the surface of the sediment. Animals hidden within the sediment quickly responded and many palp tips emerged. Such an immediate response to food was also noted by Jones (1968). In addition, the response of *M. johnstoni* was more marked when food particles were mixed with flocculent material collected from the sediment surface at the Northumberland sampling site. However, no reaction to food was observed in *M. mirabilis*, and its palps remained still within the water column, despite several foods being tested.

We observed some slight differences in feeding between *M. johnstoni* (fig. 4A–F) and *Magelona* sp., as described by Jones (1986). For *M. johnstoni*, the distal tips (one-sixth) of the palps were looped outside the burrow to collect food particles from both the sediment surface and within the water column. One palp tip raked the surface, sometimes resuspending food particles to be picked up by the other palp. Food particles were moved along the palps quickly, like a 'Mexican wave' or conveyor belt passing particles from one part of a looped palp to a more proximal adjacent part (fig. 4B). Some animals were seen to coil their palps into the burrow, bringing food within (fig. 4C) and accelerating particle transfer. Palp looping was similar to the food transfer mechanism in the account of Jones (1968), but coiling was a new observation.

In total, four different areas of food manipulation along the palp length were recognised for *M. johnstoni* (fig. 4A):

(1) The emergent distal looping/coiling zone—where food particles were moved along the palps by large movements (fig. 4B, C).

(2) The second zone, just below the surface, where each palp diverged within the sediment—food was transferred by very small loops of the palps in conjunction with direct movement by the papillae (figs 1E, 5A). Here, food particles were passed from papilla to papilla along each palp (fig. 4D).

(3) The third zone, further down the burrow, where the two palps aligned together in parallel—food particles were moved cooperatively between the papillae of both palps (fig. 4E). Particles could be moved using papillae of just one palp (as in zone 2), but the cooperative method predominated.

(4) The drop zone, coinciding with the non-papillated regions of the palps—food particles descended directly from the rapidly splayed palps to the mouth (fig. 4F). As they neared the mouth, a 'gulping' action aided consumption of the particles, and as transport of food particles within this zone was noticeably swift, it was likely that 'gulping' also created a current inward toward the mouth. No other forms of current generation (e.g. from pouch/lamellar movements, or lateral body movements) were observed during feeding.

Once consumed, food particles were readily observed through the body wall and moved rapidly through the thorax. However, the thoracic gut transition time increased as more food was consumed. McMahon and Jones (1967) and Jones (1968) postulated that there might be a mucus thread aiding the transport of food particles across the non-papillated region of the palps. They identified probable mucus-secreting cells at the bases of the papillae, along the proximal 20% of the palps and on the ventral surface of the prostomium. Jones (1968) also witnessed a coordinated movement between food particles moving to the mouth and along the gut, adding credence to the involvement of a mucus thread. However, we found no evidence for a mucus strand for *M. johnstoni*. Further, movement of particles through the thorax slowed as feeding progressed, yet movement along the palps continued as before. Food abundance had a major influence on feeding. For instance, if a glut of food was present at the sediment surface, animals continually brought food particles into the burrow, storing them just above the prostomium. One individual filled its entire burrow from prostomial tip to sediment surface with food, which was later fed upon more slowly. Despite burrows being temporary structures, the burrow structure around the palps was well maintained during feeding.

On occasion, individuals emerged slightly from the sediment and moved their palp tips towards the mouth region. This occurred simultaneously with 'proboscis' eversion that created a channel in which the palp tip was wiped across the mouth. The function of this behaviour was unclear.

Magelona mirabilis did not feed in the same manner as *M. johnstoni*. Its palps remained erect within the water column and made no response to the addition of food. Only relatively small movements of the palps were observed, even when water flow was increased. The species never waved its palps in the water column, as seen in *M. johnstoni*, even under comparable flow rates.

The palps of *M. mirabilis* (fig. 2A) (particularly the proximal third) were extremely stiff in comparison with those of *M. johnstoni*, even in relaxed animals. The blood vessels within the palps (compare figs 1F, G with 2B, D) were clearly much wider in *M. mirabilis*, and this may contribute to their rigidity. The general 'feeding' position within the burrow was similar to that of *M. johnstoni*, though *M. mirabilis* kept its palps closer together (fig. 7), the papillae (fig. 2C) of each extensively interlocked.

Then, on day 63, two animals were seen to consume large amounts of sediment, ignoring the recent addition of food. Their palps remained stiffly displayed within the water column, however long thin 'pellets' of sediment were brought down to the non-papillated region of their palps. These pellets were possibly formed as sediment was squeezed between the interlocked palps, but this could not be confirmed as the burrows were only partially visible. On several occasions, individuals were observed to move their palps backwards and forwards in a saw-like motion. Pellet movement was rather slow and often jerky, particularly in the region of the prostomium. This movement was unlike that observed for *M. johnstoni*. Again, no evidence of a mucus string was apparent, although sediment particles moved as if 'tugged' towards the mouth. Consumption of sediment was slow, with no quick 'gulping' action, and material built up around the mouth region. The 'mouth' region of *M. mirabilis* seemed to be more extendable than that of *M. johnstoni*, protruding more as a tube as it gathered in particles.

Apart from the apparently infrequent sediment ingestion, another possibility was that *M. mirabilis* preferentially fed on minute particles (and/or bacteria) in the water, which were unable to be seen using the techniques utilised here. This could explain why the papillae of its palps within the burrow were so interlocked. There was no evidence that the species employed a form of mucus-net suspension feeding, as found in certain other polychaetes (Riisgård and Larsen, 2010), though debris was seen to become lodged in between and along the length of the palp tips of *M. mirabilis* (fig. 7). All experimental individuals of this species survived over seven months within the tank. Further investigations with an increased number of animals are needed before any conclusions about its mode of feeding can be made.

Palps of *M. johnstoni* appear to be selective in what they pick up, using the papillae at the palp tips like fingers. Selectivity of the magelonid diet has been previously suggested (Hunt, 1925; Linke, 1939), and Fauchald and Jumars (1979) considered the group selective surface deposit feeders, and that selectivity may increase within poorly sorted sediments. However, the possibility of suspension feeding has been suggested by other authors (see Rouse, 2001). Our observations have shown *M. johnstoni* to both capture particles suspended within the water column as well as from the sediment surface, an idea previously suggested for *M. papillicornis** (Wolff, 1973; Hartmann-Schröder, 1971), thus implying two different feeding modes.

The constituents of the magelonid diet have been noted by several authors (McIntosh, 1911; Hunt, 1925; Mare, 1942; Jones, 1968; Hartmann-Schröder, 1971; Wolff, 1973; Kühl, 1974) and include detritus, diatoms, organic debris, algal cysts, spores, foraminiferans, tintinnids, and the larvae of

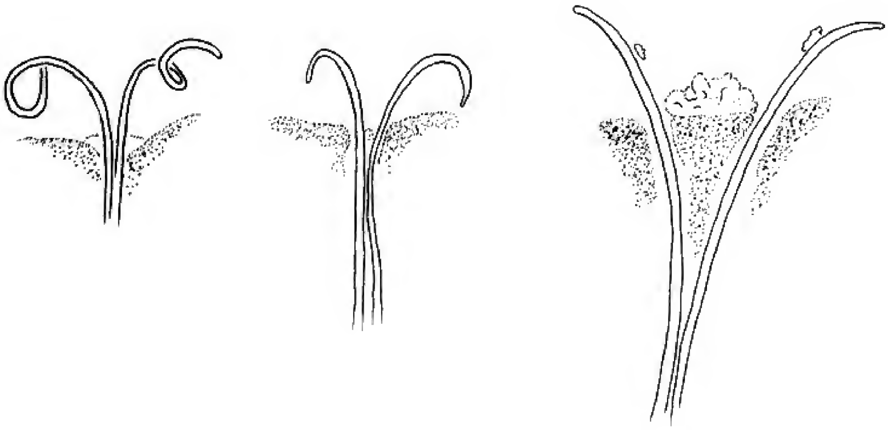

Figure 7. Various palp positions observed for *Magelona mirabilis* from *in situ* experiments. Third picture depicts debris collecting on and in between the palps.

crustaceans, molluscs and worms. Bivalve and polychaete larvae, pelagic eggs and tintinnids have been recorded in the diets of magelonid larvae (Lebour, 1922; Thorson, 1946; Smidt, 1951; Kühl, 1974; Wilson, 1982). Although doubts exist as to whether natural predation on bivalve larvae is common (Johnson and Brink, 1998), prevalence may be higher in later-stage larvae (Wilson, 1982; Johnson and Brink, 1998).

McIntosh (1911) and Mare (1942) additionally reported the presence of sand, silt and debris, which likely concurs with our observations for *M. mirabilis*, and lends support for the presence of interspecific variation in feeding between co-existing magelonid species. This, and contributing factors such as behaviour, size, morphology, habitat, presence/absence of a tube, and palp morphology (e.g. stiffness/flexibility), warrants further investigation.

Palp regeneration

Several *M. johnstoni* lost their palps upon collection, and this provided an opportunity to monitor their regeneration. All animals initially stayed well within the sediment and were not visible for the first few days. By day 3, one individual began protruding the tip of its prostomium out of the sediment surface, occasionally everting its 'proboscis'. This individual moved fairly swiftly around the tank, repeating this behaviour in different locations. While at the surface, it created an inward current toward the mouth by combining 'gulping' with everting and retracting the 'proboscis', enabling feeding despite the loss of palps. Capillary tube experiments confirmed that conspicuous inward currents could be produced in this manner.

Palp regeneration progressed at different rates between animals, and between palps on the same individual (table 1). By day 29, one pair of palp tips were noticeable protruding out of the sediment surface (palps now up to nine times prostomial length), and by day 31, one animal was using its palps to feed. Although these palps were thinner and shorter than those in intact animals, they were able to manipulate food particles effectively and bring food to the mouth, as previously described for the species (see above). McIntosh (1911) described the rapidity by which magelonids regenerated their palps, noticing that within 3 days "the new organs appeared on each side as short blunt processes into which the blood entered". This agrees well with the current observations, which saw short stumps on every animal within the same period.

During regeneration, individuals were seen to carry out lateral sinuous movements of the thorax within the burrow. This was first noticed on day 3 and continued sporadically up until day 24. The purpose of this behaviour was unclear, however, magelonid palps are thought to also have a respiratory function; this will be discussed more fully below.

Observations were also made on individuals that had lost only one palp. Initially, such animals were observed to stay close to the sediment surface, with the remaining palp extended into the water column and waved, as in normal behaviour. Palp regeneration followed a similar time-scale to that of animals lacking both palps. By day 8 they were a third of the length of the prostomium, and by day 18, about twice the length of the prostomium. By day 36, one regenerating palp was of similar length to the intact palp (less than one prostomium length's difference in size). Single-palp individuals collected food particles effectively with their remaining palps, using a similar technique to that seen in zone 2 of intact animals (fig. 4D). Although transfer of food particles was slower, their feeding capability did not appear compromised in any other way.

These observations suggest the implication of palp loss in magelonids is lessened by their ability to continue to feed either with only one or no palps, and their ability to regenerate to fully functioning palps within ~30 days.

Sinuous lateral movements

On occasion, *M. johnstoni* made gentle, sinuous lateral movements of the thorax within the burrow, often for long periods. Jones (1968) also observed movements of the anterior 20 to 25 chaetigers of *Magelona* sp. (~85–100 times per

Table 1. Showing palp regeneration data for three *Magelona johnstoni*, all of which lacked palps on collection.

Day	Palp length (in prostomium lengths)			Notes
1–2	–	–	–	All animals within the sediment
3	Stumps noticeable	Stumps noticeable	Stumps noticeable	One prostomium protruding just out of the sediment. Very slight lateral movements of body observed.
4	1/6	–	–	All at or near sediment surface.
8	1/3	–	–	
10	2/3	–	1/6	All animals within the sediment; one just below the surface. Palps regenerating at different rates.
16	2	2	1/3	Two animals making slight lateral movements within burrow. Animal with shortest palps protruding prostomium tip just out of sediment.
17	–	–	–	One animal continuing small lateral movements of the thorax within the burrow. Another protruding prostomium tip just out of sediment.
21	–	–	3/4	One animal at surface with prostomium tip just emerging from sediment, while another was undergoing lateral movements of the thorax within burrow.
22	–	4, 1½	–	Unequal regeneration of palps occurring on one animal. One animal making lateral movements within burrow.
23	–	–	–	No animals at surface or undergoing lateral movements.
24	–	–	–	One animal making lateral movements.
29	–	–	–	First observation of palps emerging from the sediment surface. Palps relatively thin.
30	~9	–	2	Palps appearing equal in length.
31	~9	–	–	Palp tips at surface, first observation of an animal feeding using their palps, although palps still relatively thin.
35	~12	–	–	Palp tips within water column.
36	–	8, 2	–	Palp regeneration uneven within the same animal.

minute), which produced an inward-moving current. Jones noted that this behaviour was infrequent and postulated that it was linked to respiration, as occurrence and intensity of this behaviour increased if the water was allowed to become deoxygenated. Oxygen levels in our study were always maintained and we could not confirm this. However, sinuous movements were seen more frequently in individuals regenerating palps.

Both Jones (1968) and McIntosh (1911) believed that magelonid palps had, in part, a respiratory function, on the basis of their vascular nature and their placement into the water column. Additionally, when the current within the tank was halted, individuals extended their palps further into the water, holding them stiffly upwards. Therefore, a higher occurrence of these sinuous movements in individuals lacking palps could be a compensatory response for a loss of respiratory capacity. The relationship between burrow irrigation and body undulations has also been reported for other annelids. For example, Wells (1949) stated that *Arenicola marina* Linnaeus, 1758 irrigated their burrows, providing a supply of oxygenated water, using special waves that travelled along their bodies, usually from tail to head. Female *A. marina* could also use an altered form of this irrigation behaviour during reproductive events (Hardege and Bentley, 1997), bringing sperm into the burrows.

Lateral movements also occurred outside of the burrow. These movements were brisk and quite marked, with the sides of the body almost touching the sediment on both sides, in contrast to the gentle undulations seen within the burrow. McIntosh (1911) stated that magelonids protrude their anterior region from the sand into the water column for aeration and food, suggesting that the modified chaetae of the 9th chaetiger aided emergence from the burrow. However, he made no mention of lateral movements of the body in conjunction with this behaviour, unlike Jones (1968) who noted this behaviour in individuals without palps, believing it related to respiration. This behaviour occurred "even when there appeared to be an adequate supply of fresh, oxygenated sea water". This is in contrast to the current findings, as individuals without palps generally remained within the sediment, only bringing their prostomial tips above the sediment surface, and these movements occurred in individuals with intact palps and in aerated water, suggesting no link to respiration.

During lateral movements, the lateral abdominal pouches were generally kept flat against the body wall, and only slight

movements in response to body movement were observed. While no sinuous lateral movements of the body were observed in *M. mirabilis*, further investigations with an increased number of individuals are warranted.

Reproductive behaviour?

As stated above, animals periodically extended their thoraxes out of their burrows/capillary tubes, generally to the thorax/abdominal junction (but occasionally to approximately chaetiger 15–20). Individuals would then display lateral sinuous movements of the thorax outside of the burrow, with sporadic eversion of the 'proboscis'. These out-of-burrow movements often lasted for long periods of time, unless the animal was disturbed. However, this was an intermittent behaviour and generally took place during the months of April to July. No instances of this behaviour occurred after this period. Lateral movements were witnessed in animals with both palps intact, and occurred in both still and flowing, aerated water. Movements were often observed simultaneously in several animals, and during these periods pairs would often lean towards each other sometimes with bodies in direct contact (fig. 8).

During one observation in an isolated tank with slightly raised water temperature, an individual emerged from its burrow and commenced sinuous lateral movements of the thorax, directed towards a second individual believed to be female. Individual two was just below the surface, but the palps of each were in direct contact with those of the other. This occurred for several minutes before individual two retracted into its burrow, later emerging in another burrow some 3.5 cm away. Individual one emerged to the approximate level of chaetiger 15, stretching across the sediment towards the new position of individual two. Individual two appeared to respond to these lateral movements, by waving and looping the palps towards individual one. Individual two remained within the burrow, just below the sediment surface, but both individuals commenced entwinement of their palps until the tips became quite interlocked. After a period of time, individual two emerged from the burrow, to the approximate level of chaetiger 5. Cessation of palp entwinement occurred and individual two disappeared back into its burrow. Although the exact reason for this is unclear, the individual may have responded to vibrational stimuli. Individual one remained on the surface of the sediment for some time, continuing to stretch towards individual two, making lateral prostomial movements and eversion of the 'proboscis'. Eventually, individual one withdrew into the sand and began burrowing in another direction. Simultaneously, in this isolated tank, another pair were observed making lateral movements of the body, one within the water column and one within the sediment. The latter individual later emerged from the burrow and continued moving the thorax outside the burrow. Four days later, a further two individuals (one female and one male) carried out lateral movements. Although, no release of reproductive products was ever confirmed, the synchronised/reactive behaviour of individuals outside of the burrow was strongly suggestive of an involvement in reproduction. The simultaneous spawning of gametes in broadcast spawners would increase the probability of egg fertilisation.

Hardege and Bentley (1997) stated that synchronicity of gamete release within a population was particularly important for semelparous species, and that environmental factors such as photoperiod, temperature, lunar periodicity and tidal cycles may help in the synchronisation of broadcast spawners (believed to be the case for magelonids, see below). The observations of synchronised movements as described above during periods of increased water temperature suggests this is an important factor triggering this behaviour in magelonids. In addition, pheromones can play a final part in synchronising reproduction in both iteroparous and semelparous polychaete species (Hardege and Bentley, 1997).

One possibility is that lateral movements of the thorax may be involved in gamete release, either helping bring sperm into the burrow for egg fertilisation, as seen in female *Arenicola marina* (Hardege and Bentley, 1997), or dispersing both eggs and sperm. Jones (1968) showed that sinuous movements of the body within the burrow of *Magelona* sp. produced an inward current, suggesting that sperm released by the male could be drawn into the burrow for egg fertilisation. However, most records suggest that magelonid eggs and sperm are spawned directly into the water, and sperm structure would suggest fertilisation outside of the burrow (Blake, 2006; Rouse, 1999, 2006).

Our video footage of *M. johnstoni* clearly shows an exhalent current from the burrow during lateral movements outside the burrow, and it seems probable that if tubes are blind-ending, any inward current should circulate around the burrow and back out. If eggs and sperm are released from the posterior end into the burrow, then circulatory currents may help to push them from the burrow into open water.

Reproduction

Eggs were observed in *M. johnstoni* collected in November 2012 (Rhossili), and March and April 2013 (Berwick-upon-Tweed), with reproductive animals appearing more fragile abdominally. Reproductive females were white abdominally, in stark contrast to the conspicuous green gut (fig. 1H). Eggs were observed in *M. mirabilis* from November 2012 (Rhossili) and April 2013 (Berwick-upon-Tweed), while they were observed in animals of *M. filiformis* collected in January, February (Oxwich Bay) and April 2013 (Berwick-upon-Tweed).

Wilson (1982) collected mature eggs from *M. mirabilis** between May and August, although the best fertilisations occurred in animals from July and August. This was in agreement with McIntosh (1877), who stated that *M. papillicornis** was full of ova and sperm at St Andrews in June, and "the ova and spermatozoa … attain perfection in summer and autumn". McIntosh (1911) stated that ova of a considerable size were present in large numbers at the end of June, but those that developed in late autumn did not successfully produce embryos. Kühl (1974) suggested that *M. papillicornis** in Elbe, Cuxhaven, Scharnhörn and Gelbsand reproduced during the summer months. Wilson (1982) considered the spawning season for *M. filiformis* in Plymouth to peak during August, although mature gametes were

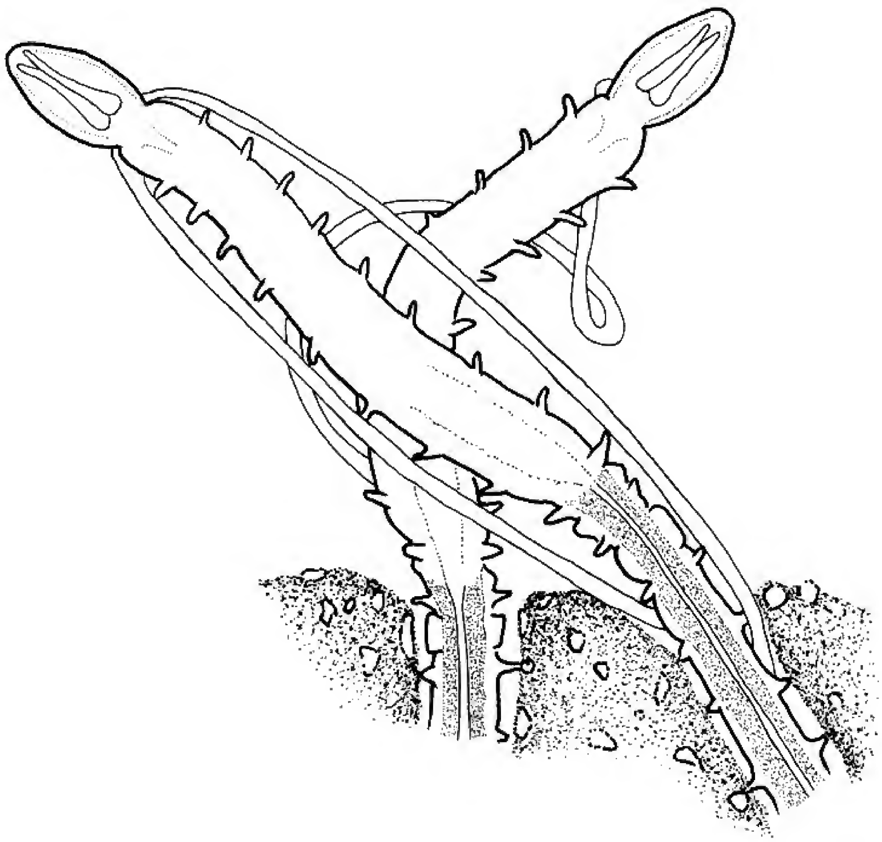

Figure 8. Showing two individuals of *Magelona johnstoni* simultaneously making lateral sinuous movements of the thorax (outside the burrow) (dorsal views).

collected between April and October, while *M. alleni* was likely to mature in late September or October.

The timing of spawning may be influenced by the availability of food. Kühl (1974) suggested that *Magelona* larvae were dependent on bivalve larvae of the right size. *Magelona* larvae were generally present in the Plymouth Sound plankton from April to November, but more commonly from July to October (Wilson, 1982). McIntosh (1915) encountered *Magelona* larvae from May–November in St Andrews, (locality not stated but assumed from McIntosh 1916: pl. XCIV, fig. 17), although numbers were generally much lower in October and November. Similar timings were noted by Kühl (1974) for *M. papillicornis** larvae, present from May to October in polyhaline rivers discharging into the German North Sea (Elbe, Weser) and the Wadden Sea (Ems). *Magelona* juveniles may settle from the plankton by September, as indicated by samples collected in bottom-nets (McIntosh, 1915).

Nevertheless, very little is known about magelonid reproduction (Rouse, 2001). They are believed to be broadcast spawners, with ect-aquasperm (as defined by Jamieson and Rouse, 1989) (Blake, 2006; Rouse, 1999; Rouse, 2006). The mechanism by which *Magelona* species shed gametes from their burrows is unknown. Several accounts have suggested magelonids only reproduce once, with mortality occurring after spawning. Ripe magelonids can be extremely fragile and, as also noted by McIntosh (1911), "it is possible that at the reproductive season degeneration of the organs [palps] may occur in some instances, or the animals themselves may perish". Fauchald (1983) considered *M. sacculata* to be an annual species (monotelic/semelparous) with feeding larvae, based partly on the work by Hannan et al. (1977) in Monterey Bay.

Species activity

Observations show that *M. johnstoni* is a very active worm in comparison with the other two species investigated. Behavioural differences were obvious: *M. filiformis* was the most inactive and *M. johnstoni* the most active. The species differ markedly in terms of body shape and size: *M. filiformis* being very slender and long, comprising of many segments, while both *M. mirabilis* and *M. johnstoni* are broader animals. *Magelona johnstoni* moved around the environment more

frequently, while *M. mirabilis*, the larger of the two species, stayed very still, inhabiting burrows for much longer periods. Additionally, *M. mirabilis* appeared much less responsive to vibrational stimuli than *M. johnstoni*, which reacted to the slightest of knocks. Jones (1968) noted that the *Magelona* sp. was also extremely sensitive to vibrational stimuli, both within the sand and in the water column, and stated that its lateral organs (see figs 5 and 10) shared similarities with those vibration receptors found in ctenophores and chaetognaths. Mucus production in *M. mirabilis* was much lower than in *M. johnstoni* during observations. *Magelona johnstoni* placed in petri dishes with a small amount of sediment were shown to cover themselves in a mucus/sediment mixture very quickly, producing a rudimentary 'tube'. McIntosh (1915) noted this behaviour, suggesting that this is "probably to compensate for the absence of its element".

Pouches

The most obvious morphological feature separating *M. johnstoni* and *M. mirabilis* is the respective presence or absence of anteriorly opening abdominal lateral pouches on the anterior abdomen. The function of these (and other posteriorly directed pouches) in magelonids has never been resolved, despite much attention (McIntosh, 1878, 1911; Jones, 1968).

No significant movements of lateral abdominal pouches, either anteriorly or posteriorly opening forms, were observed for *M. johnstoni* during any capillary tube or *in situ* experiment. In general, the pouches were kept flat against the body. Any slight contractions of the anteriorly opening pouches were attributable to body movements. Lateral movements of the thorax would cause the lateral edges of the dorsal and ventral flaps to come together, and when animals lunged forward, pouches occasionally contracted slightly as the body elongated and narrowed. Slow-motion video footage also revealed small pouch contractions as the lumen/ventral vessel of the posterior region contracted, often propagating a wave down the abdomen. On occasion, the first pair of anteriorly opening pouches expanded against the sides of the capillary tubes, but this was generally restricted to a few individuals in poor condition.

Water flow throughout the capillary tube was produced by eversion and retraction of the 'proboscis', and through lateral movements of the body. Observations using carmine particles showed that water movement around the pouches was not significantly greater than that around parapodia and segments of other parts of the body. Water flowing along the dorsal and ventral edges of the body was directed laterally around parapodia and toward the opening of the pouches (fig. 9). No additional flow created by pouch function was evident when water flowed back out.

Possible function of abdominal lateral pouches

Anchor

One hypothesis is that pouch function may be related to anchorage, particularly during lateral body movements outside the burrow, from which *M. johnstoni* emerges to the approximate level of chaetiger 9 (just above the first pair of abdominal pouches). Pouches expanded against burrow sides could help prevent worms being swept away by water movements. However, healthy *M. johnstoni* kept their lateral pouches flat against the body (fig. 6). In addition, burrow entrances become widened during this behaviour, making such anchorage unlikely. Posteriorly opening pouches were also kept flat against the body and were never shown to expand against the burrow sides.

Propulsion

Another hypothesis is that the contraction of anteriorly opening pouches could aid movement backwards (perhaps enhancing rapid retraction when under threat of predation), with posteriorly opening pouches enabling movement forwards. No evidence of pouch contraction could be seen in video of *M. johnstoni* for either slow or rapid movement, forwards or backwards. The presence of a medial slit in posteriorly opening pouches in some species also suggests that this is an unlikely function.

Reproduction, sperm storage, and brooding

Throughout this study, gametes were present within the body cavities of *Magelona* specimens, but no relationships between gametes and pouches were observed. Jones (1968) doubted any relationship between pouch function and reproduction because of their presence in both sexes and in juvenile forms. Conversely, McIntosh (1877, 1878, 1879) believed that 'lateral organs' (see below) appeared in ripe animals in summer and autumn. However, it is likely that he was examining two different species, *M. mirabilis* and *M. johnstoni*. As magelonids are thought to be broadcast spawners with ect-aquasperm, the likelihood that pouch function is related to sperm storage is low. Although sperm storage has been described for some members of the Spionida (see Blake, 2006), such as *Streblospio benedicti* Webster, 1879, *Pseudopolydora kempi* (Southern, 1921), *Pseudopolydora paucibranchiata* (Okuda, 1937) and *Pygospio californica* Hartman, 1936, sperm receptacles in these species differ greatly in morphology and show clear connections to the interiors of the animals concerned. No such connections have been found in magelonid pouches. Fauchald (1983) stated it unlikely that *M. sacculata* (a species with paired anteriorly opening pouches in the anterior abdomen) brooded its young due to its large reproductive effort. Nevertheless, pouch function could be seasonal, and without direct observation of spawning events, the link between the two cannot be completely refuted.

Burrow irrigation

Our observations suggest that magelonids use lateral movements of the thorax within the burrow (rather than contraction and expansion of pouches) to generate water circulation.

Morphology of pouches

Investigation of pouch morphology along the body of *M. johnstoni* supported a graduation between anteriorly and posteriorly opening pouches, as reported by Mortimer (2010). Understanding the form of the anteriorly opening pouches has been extremely difficult due to their apparently complex

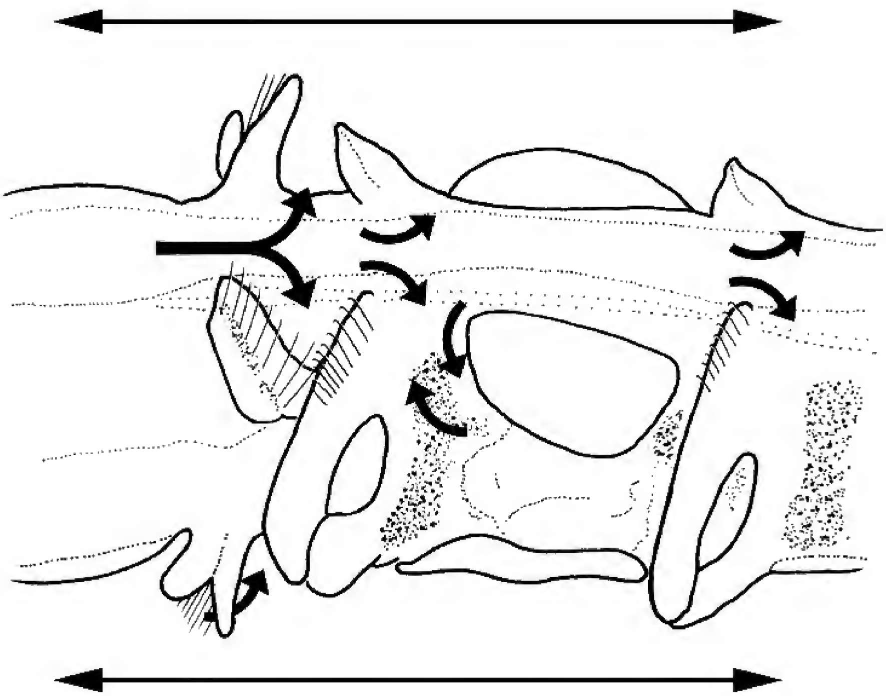

Figure 9. Lateral view of *Magelona johnstoni* between chaetigers 9 (to the left of the picture) and chaetiger 11 (to the right), showing anteriorly opening abdominal pouches between chaetigers 10 and 11. Arrows indicate water flow around the pouches and lamellae, as observed during capillary tube experiments.

convolutions. SEM images (fig. 5E–F) of transverse sections have now shown these to be simpler bags with highly convoluted surfaces. The convolutions were much greater on the external surfaces than on the internal ones, and the membranes themselves were relatively thick. No connections between pouches and the interior body cavity were apparent. The inner surfaces of the posteriorly opening pouches seemed somewhat convoluted as well (fig. 10D–E). The C-shaped flap, when viewed from a posterior direction, showed the dorsal and ventral portions to be thicker, with a thinner more textured section in between, revealing a closer affinity with the structure of the anteriorly opening forms. At the extreme posterior end, it was sometimes possible to see a small 'hole' (fig. 10A–B) at the intersegmental margin, which from the study of other partially formed pouches in the region (fig. 10C), we believe represented the initiation of a new pouch.

Pouch distribution

A review of current knowledge of lateral pouches within the Magelonidae (appendix) revealed several distinct species-groups:

1. Species without pouches (excluded from appendix, but note pouch absence may be incorrectly reported in some species).
2. Species possessing both anteriorly and posteriorly opening pouches, such as *M. johnstoni* (N.B. species for which the presence of posteriorly opening pouches is unknown are highlighted).
3. Species possessing posteriorly opening pouches on consecutive segments.
4. Species with posteriorly opening pouches on alternating segments. Pouches are generally unpaired and alternate from one side of the body to the other. Some species may have a few consecutive pouches, amongst the alternating ones.
5. Species with posteriorly opening pouches in the latter part of the abdomen only.

In groups 3 and 4, pouches are generally present from chaetigers 20–45. However, in group 5 (perhaps those species attaining the greatest number of chaetigers), pouches do not appear until the extreme posterior (i.e. approximately chaetiger 60–80 or later). Groups 3–5 are distinguishable, purely based on the pattern of pouch location, i.e. unpaired/paired and the chaetiger on which pouches are first present. However, further differentiation could be made based on pouch morphology, e.g. separating those species with medially slit pouches (usually occurring in those with paired pouches on consecutive segments).

The chaetiger on which anteriorly opening pouches first occur differs between species, the majority commencing between chaetigers 11 and 12, but some starting from chaetiger

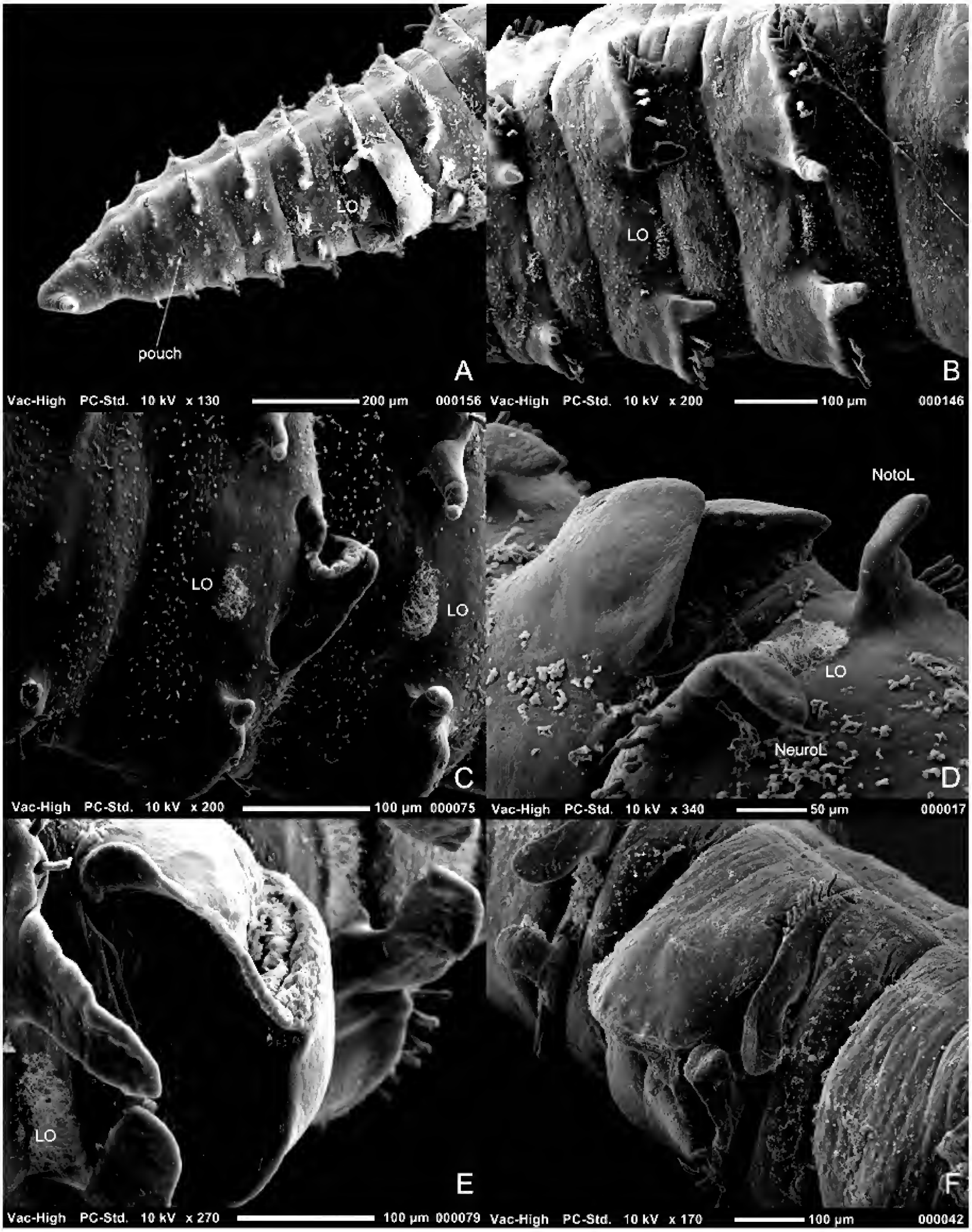

Figure 10. Abdominal posteriorly opening pouches from several specimens of *Magelona johnstoni*: A–B, initiation of new pouches represented by small 'holes' (lateral view) (A: NMW.Z.2013.037.0008e; B: NMW.Z.2013.037.0010d); C, first pouch (~6 chaetigers) from pygidium (lateral view) (NMW.Z.2013.037.0011d); D, first pouch from a regenerating tail (ventral/posterior view) (NMW.Z.2013.037.0008c); E, third pouch (~10 chaetigers) from pygidium (posterior view) (NMW.Z.2013.037.0011d); F, posteriorly opening pouch of an abdominal fragment (lateral posterior view) (NMW.Z.1998.028). Photos: K. Mortimer.

10 (chaetiger 9 is even reported in a small number of species). Most anteriorly opening pouches are paired; however, unpaired pouches are reported in some species, and this warrants further investigation, as does their number (some species only have one pair, while in others there are several). At present, details on the morphology of pouches in described species are insufficient to be able to further categorise the groups.

'Lateral organs' of McIntosh

Jones (1968) stated that the structures termed 'lateral pouches' were equivalent to the 'lateral organs' of McIntosh (1879, 1911, 1915). However, according to McIntosh's accounts (1877, 1879), lateral organs appeared in ripe individuals, suggesting a connection with reproduction. In his 1877 account under a section headed 'Reproductive Organs', McIntosh states "the ova and spermatozoa are present in each sex in great abundance in the posterior region of the body, and attain perfection in summer and autumn. On the sides of the body, also, peculiar convoluted organs occur in processes composed of the cuticle, hypoderm, and basement-tissue". Similarly in 1879, McIntosh writes "and in a male loaded with spermatozoa at the same season, and in which the lateral organs were present, the diaphanous tapering tips were extended forward nearly to the cuticle, and curved inward like the horns of the springbok". McIntosh (1879) suggested that the appearance of 'lateral organs' caused 'a curious change', in which cephalic vessels became abbreviated and the direction of blood flow at the base of the prostomium was modified, further stating that there was a greater diversity in cephalic vessels in animals bearing 'lateral organs'. McIntosh does not refer to lateral organs in his 1911 account, but does note 'peculiar structures' that occur on either side of the body in males and females with developed sexual products, on many of the posterior segments. Curiously, he states that these structures invariably occur on the segment immediately behind the mouth, stating: "and in this it first attains perfection". 'Lateral organs' are figured in McIntosh (1878: pl. XXX, fig. 7) and clearly show anteriorly opening paired pouches located between the 10th and 11th chaetigers. Also figured, is a transverse section through the body wall and 'lateral organ' (pl. XXXIV, fig. 2) from the anterior abdominal region, which shows a dorsal and ventral flap with convoluted membrane. There is some doubt about which species McIntosh studied: although originally identified as *M. papillicornis*, most European records have been referred to either *M. johnstoni* or *M. mirabilis*. Fiege et al. (2000) reviewed specimens collected by McIntosh at St Andrews, referring them to *M. mirabilis*, and McIntosh's 1916 drawing certainly shows an anterior abdomen lacking anteriorly opening pouches. Yet, the pouches drawn by McIntosh (1878) are indicative of *M. johnstoni*, although no locality was given for this particular specimen. McIntosh (1915) stated that "on the sides of the posterior region, from the twenty-fifth or twenty-sixth segment backward, are the peculiar glandular organs (pouch-like) which occupy the lateral region of each segment". Abdominal pouches do not occur in *M. mirabilis* until approximately chaetiger 80 (see Fiege et al. 2000: 226 and Appendix), but posteriorly opening pouches are present in *M. johnstoni* from around chaetiger 20. As these two species were not differentiated until 2000, it is extremely likely that McIntosh was observing the two morphologically similar and co-existing species *M. johnstoni* and *M. mirabilis* under the name of *M. papillicornis*. Hence, the occurrence of 'lateral organs' was actually an unrecognised species-specific character, and not related to reproduction. Although McIntosh did not always state the location of specimen collection, references to St Andrews throughout his accounts exist (1877, 1878, 1911, 1915), and text clearly states that specimens possessing 'lateral organs' were present alongside specimens without.

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Appendix.

All known information from literature records on the presence, morphology and pattern of lateral abdominal pouches within the Magelonidae. Key to categories: 1) species in which pouches are reported as absent (not included in table); 2) species possessing both anteriorly and posteriorly opening pouches (presence of posteriorly opening pouches unknown/not confirmed in some species+); 3) species with paired posteriorly opening pouches on consecutive segments; 4) species with unpaired posteriorly opening pouches alternating from one side of the body to the other, on alternate segments (sporadic pouches on consecutive segments may be present); 5) species with posteriorly opening pouches in the latter part of the abdomen only.

Species	Notes	Chaetiger of first appearance	Record	Category
Magelona sp. of Jones (1968)	Paired anteriorly opening pouches between chaetigers 10 & 11.	10	Jones (1968)	2
	Unpaired pouches, approximately every 4th chaetiger, alternating from one side of the body to the other.	20–23		
Magelona sp. A	Large, paired pouches.	11	Uebelacker and Jones (1984)	2
	Posteriorly opening pouches, unpaired on alternate chaetigers, on alternating sides of the body.	26		
	*Notes only paired and unpaired C configuration pouches.		*Brasil (2003)	
Magelona sp. B	Large, paired pouches.	11	Uebelacker and Jones (1984)	2
	Posteriorly opening pouches, unpaired on alternate chaetigers, on alternating sides of the body.	18–26		
	*Notes only paired and unpaired C configuration pouches.		*Brasil (2003)	
M. cincta Ehlers, 1908	C configuration, unpaired on alternate chaetigers and alternate sides of the body, based on a single specimen from Mozambique. Not observed on holotype (specimen short anterior fragment).	19	Mortimer and Mackie (2009)	4
M. conversa Mortimer & Mackie, 2003	Σ configuration, paired (11, 14, 17, 20). Unpaired pouches on alternate chaetigers and alternate sides of the body.	11	Mortimer and Mackie (2003)	2
	*Several unpaired pouches very large, more akin to Σ configuration pouches.	23–26	Mortimer et al. 2012*	
M. cornuta Wesenberg-Lund, 1949	C configuration, paired, on consecutive segments, medially slit, edges of which are surrounded by thicker cuticle (based on specimens from Hong Kong, not observed on short holotype).	~41	Mortimer and Mackie (2009)	3
M. crenulata Bolívar & Lana, 1986	Bolsas genitais pareadas no setígero 11 e não pareadas nos setígeros 20 e 28 [paired genital bags on setiger 11 and unpaired on chaetigers 20 to 28].	11; 20–28	Bolívar and Lana (1986)	2?
	*Paired and unpaired Σ configuration.		*Brasil, 2003	
M. crenulifrons Gallardo, 1968	C configuration, unpaired, on alternate chaetigers and alternate sides of the body. Not originally described, but present on type material.	25* based on Hong Kong specimen	Mortimer and Mackie (2009)	4

Species	**Notes**	**Chaetiger of first appearance**	**Record**	**Category**
M. dakini Jones, 1978	Unpaired, alternating from one side of the body to the other, irregularly located on chaetigers.	101–117	Jones (1978)	5
	*Unpaired C configuration pouches.		*Brasil (2003)	
M. debeerei Clarke et al., 2010	∑ configuration, paired between chaetigers 10 & 11 and 14 & 15, unpaired pouches present between 13 & 14 in some specimens. C configuration not observed	10	Clarke et al. (2010)	2?
M. filiformis Wilson, 1959	C configuration occurring at the extreme posterior end, unpaired, on alternate segments and alternating from one side of the body to the other. Not recorded in original description and reported as absent in Fiege et al. (2000) and Brasil (2003).		This study	5
M. gemmata Mortimer & Mackie, 2003	C configuration, paired, on consecutive segments	42	Mortimer and Mackie (2003)	3?
Magelona sp. G	Posteriorly opening pouches, paired, on consecutive segments.	27–28	Uebelacker and Jones (1984)	2/3?
	*Paired, ∑ configuration pouches present.		*Brasil (2003)	
M. hartmanae Jones, 1978	Unpaired, initially on alternate segments and alternate sides of the body. However, variation in pattern occurs more posteriorly. Occasional pouches on consecutive segments.	42–48	Jones (1978)	4
	*Unpaired C configuration pouches present.		*Brasil (2003)	
M. heteropoda Mohammad, 1973	∑, paired, *membrane on both sides of holotype presumed missing.	11	Mohammad (1973), synonymised with *M. obockensis*, see Mortimer (2010)	2
	C configuration, unpaired, more or less alternating between chaetigers and side of the body. Pouches quite large, expanded more dorsally and ventrally, often convoluted.	17		
M. johnstoni Fiege et al., 2000	∑, those between 10 & 11 paired, then several pouches occur either paired or unpaired. Some variation in patterns.	(9) 10	Fiege et al. (2000)	2
	C configuration, unpaired, *on alternate segments and alternating sides of the body.	~20	*Present study	
Magelona sp. L	Posteriorly opening pouches, paired on consecutive segments.	28–31	Uebelacker and Jones (1984)	3
	*Paired C configuration pouches.		*Brasil (2003)	
M. lusitanica Mortimer et al., 2011	Unpaired posteriorly opening pouches, alternating from one side of the body to the other, either on consecutive segments or every other. Pattern varies along body.	36	Mortimer et al. (2011)	4
M. mahensis Mortimer & Mackie, 2006	Unpaired C configuration pouches present, on alternate chaetigers, on alternate sides of the body, "Often more or less folded, with thicker cuticle on edges of fold and thinner cuticle inside. Edges of fold can be abutting or overlapping."	38	Mortimer and Mackie (2006)	4

Species	Notes	Chaetiger of first appearance	Record	Category
M. mirabilis, (Johnston, 1865)	C configuration pouches present, paired, occurring on every 3 or 5 segments for the neotype.	~78	Fiege et al. (2000)	5?
	*Paired C configuration pouches present.		*Brasil (2003)	
M. montera Mortimer et al., 2012	Posteriorly opening, paired pouches on consecutive segments. "Pouches appear as simple folds, split medially with thicker cuticle surrounding edges".	38	Mortimer et al. (2012)	3
M. obockensis Gravier, 1905	∑, paired between chaetigers 11 & 12. Unpaired, anteriorly opening pouches present on one specimen, more closely resembling posteriorly opening pouches.	11	Mortimer (2010); Gravier (1906)	2
	C configuration pouches, unpaired, alternating from one side of the body to the other, usually on alternate segments, "often quite large, more expanded both dorsally and ventrally, often convoluted". Mortimer (2010) suggested this represented a graduation between the two pouch morphologies along the body.	17–27[1]	[1]Based on senior author's notes on syntype material	
	*Only paired C configuration pouches present.		*Brasil (2003)	
M. pacifica Monro, 1933	Paired posteriorly opening pouches, on consecutive segments. Medially split, with thicker cuticle surrounding edges.	36–40	Mortimer et al. (2012)	3
M. parochilis Zhou & Mortimer, 2013	Paired, anteriorly opening pouches between 11 & 12 and 14 & 15 (occasionally between 17 & 18).	11	Zhou and Mortimer (2013)	2
	Unpaired posteriorly opening pouches, on alternate chaetigers, alternating from one side of the body to the other.	21		
M. pectinata Nateewathana & Hylleberg, 1991	Large lateral pouches usually present between chaetigers 11 & 12 and 13 & 14. Other records of pouches present between chaetigers 10 & 11 and 12 & 13, 20 & 21, and 23 & 25. Smaller sporadic pouches are recorded for the posterior[+].	10/11	Nateewathana and Hylleberg (1991)	2?
M. pitelkai Hartman, 1944	Posteriorly opening, unpaired, on alternate segments, alternating from one side of the body to the other.	64–84	Jones (1978)	5
M. pulchella Mohammad, 1970	C configuration, initially alternating and unpaired, then paired on consecutive segments. In the posteriormost region they are unpaired on consecutive segments, alternating from one side to the other.	39	Mortimer (2010)	3/4
M. rioja Jones, 1963	Pouches present in posterior region, occurring in a sporadic and irregular pattern; they "appear to be identical with similar structures described by Hartman (1961) for *Magelona sacculata* and others".		Jones (1963)	2?
	*Paired and unpaired ∑ configuration pouches.		*Brasil (2003)	
M. sacculata Hartman, 1961	"Conspicuous pouched membranes, first present behind the modified ninth segment, occur also between segments 10 and 11, and at irregular intervals in abdominal segments". Note: original figure only shows pouches between segments 10 and 11 (paired).	9/10?	Hartman, 1961	2?
	*Paired and unpaired pouches of both morphologies present.		*Brasil (2003)	

Species	Notes	Chaetiger of first appearance	Record	Category
M. sachalinensis Buzhinskaja, 1985	Large paired pouches between chaetigers 11–12 and further irregular pouches, which may occur either singly or paired+.	11	Buzhinskaja (1985)	2?
M. tinae Nateewathana & Hylleberg, 1991	∑, paired.	11	Nateewathana and Hylleberg (1991)	2
	C configuration, unpaired, roughly on every other segment, alternating between sides of the body. Pouches quite large, often convoluted.	22	Mortimer (2010)	
M. wilsoni Glémarec, 1966	Posteriorly opening, unpaired, alternating from one side of the body to the other. Pattern irregular, sometimes on consecutive segments, sometimes alternately (description based on a Gulf of Lions specimens, not observed in type material).	24	Mortimer et al. (2011)	4

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The identity of juvenile Polynoidae (Annelida) in the Southern Ocean revealed by DNA taxonomy, with notes on the status of *Herdmanella gracilis* Ehlers *sensu* Augener

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Abstract

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Using molecular data (CO1, 16S and H3 genes), we provide evidence for a long-held view that Southern Ocean scaleworms (Polynoidae) morphologically agreeing with *Herdmanella gracilis* sensu Augener, 1929 Ehlers sensu Augener are in fact juveniles of another species. The problematic genus *Herdmanella* is declared a *nomen dubium*. Importantly, at least two species were identified; one adult counterpart is a common circumpolar species, *Austrolaenilla antarctica* Bergström, 1916, and the other is of an as yet unknown identity. More adult counterparts are likely to be discovered with greater sequencing effort and larger taxon coverage. We have discovered a great genetic diversity within the *A. antarctica* clade in the CO1 gene, and future studies may elucidate if this represents a cryptic species. Currently, we adopt a conservative approach and suggest that given low diversity in mt16S and complete identity in H3 genes, this clade represents a single species, with only the specimen from South Georgia likely deserving the status of a cryptic species, as shown by haplotype network analysis. High mtDNA diversity in populations of Antarctic scaleworms may be linked to habitat fragmentation during recent glacial periods. Our study also highlights the importance of identifying juvenile specimens correctly in order to understand ecological processes such as the apparent high productivity in the Amundsen Sea region.

Keywords Antarctica, Polychaete, DNA barcoding, marine diversity, population genetics, deep sea, cryptic species

Introduction

Exploration of our still largely unknown oceans continues to yield new taxonomic discoveries, mostly resulting in descriptions of new species. However, increased collecting effort, combined with new molecular tools, also provides an opportunity to address longstanding problems in taxonomy. Molecular taxonomy in general, and 'DNA barcoding' (Hebert et al., 2003) in particular, has grown quickly as a discipline in the past decade. Despite important problems with this approach (e.g. Meier et al. 2008; Collins and Cruickshank, 2012; Bergsten et al., 2012; Srivathsan and Meier, 2012), DNA barcoding has become an important tool in a diverse range of biological disciplines. In taxonomy, it has been primarily implemented for problems of species identification and species delimitation (see e.g. Monaghan et al., 2006; Vogler and Monaghan, 2007; Hamilton et al. 2011). Additionally, DNA barcoding has proved successful in linking different developmental stages of the same species in a wide range of animal taxa, such as shrimps (Shank et al., 1998), beetles (Ahrens et al., 2007), marine invertebrates (Webb et al., 2006; Heimeier et al., 2010; Bracken-Grissom et al., 2012) and fish (Pegg et al., 2006; Valdez-Moreno et al., 2010).

In the marine environment, plankton and nekton collections commonly include larval and juvenile developmental stages that differ from their adult counterparts in their morphology. Early taxonomists, often unaware of the existence of the larval stages, sometimes misidentified these morphologies as independent adult lineages (Bracken-Grissom et al., 2012). Past approaches to these problems included rearing experiments in aquaria (Richards and Saksena, 1980; Haynes, 1982) or *in situ* (Haynes, 1979). However, this approach is not always practical and requires a collection of live larvae. Often, if specimens were primarily collected for other types of studies, such as biodiversity surveys, they may not have been collected live and a molecular approach therefore represents an alternative tool for identification of larval stages.

For over 100 years, the polynoid species *Herdmanella gracilis* Ehlers, 1908 presented a problem for polychaete taxonomists. Ehlers (1908) described this species upon examination of a number of specimens collected from deep water (1500–2000 m) off the coast of East Africa (in the Indian Ocean) during the *Valdivia* expedition 1898–99. It is not clear if a type specimen of this species was deposited, but no types are known to exist (Pettibone, 1976). Given the small size of this species (1.5 mm long, 15 segments, 8 pairs of elytra), it has long been suggested that it represents a juvenile form of an otherwise benthic species. Ehlers (1908) expressed some reservations: "… it is not impossible (even though in my opinion improbable) that it is a juvenile of a known species …", but proceeded with formal description anyway. His decision to assign this species to the genus *Herdmanella* Darboux, 1899 was never properly explained. Later, Monro (1930) suggested *H. gracilis* could be a juvenile stage of the polynoid *Antinoë pelagica*, now known as *Austrolaenilla pelagica* Monro (1930) from the Southern Ocean. Hartmann-Schröder (1974) considered *Herdmanella gracilis* to be the juvenile of a species related to the polynoid genus *Harmothoe*, a view supported by Pettibone (1976), who considered *H. gracilis* to be a doubtful species belonging to the subfamily Harmothoinae (now Polynoinae, see Muir, 1982). Once recognised as a valid species, Augener (1929) identified small polynoids from the Weddell Sea as *Herdmanella gracilis,* and in doing so expanded its range to the Southern Ocean, proposing that it could be a very widespread form that can presumably live in the tropical deep sea as well as the shallow high-latitude regions. This resulted in *H. gracilis* being listed in *Polychaeta Errantia of Antarctica*, an atlas compiled by Hartman (1964).

Further, the new species *Herdmanella aequatorialis* Støp-Bowitz, 1991 has been described from off West Africa (equatorial Atlantic Ocean) at 300 m depth. Støp-Bowitz (1991) recognised that, given the small size of the single, damaged specimen, it may be a juvenile, but unable to assign it to any other known genera, and given its similarity to *H. gracilis*, he proceeded with erecting a new species in the genus *Herdmanella*. The two species *H. gracilis* and *H. aequatorialis* are currently regarded as the only valid species in *Herdmanella*, although this itself is a problematic genus (its status is addressed in the Discussion section of this paper). The other species previously referred to this genus, *Herdmanella nigra* Hartman, 1967, has been transferred to *Bathyeliasona* by Pettibone 1976.

As part of the 2005 BIOPEARL I expedition to the Scotia Sea (Linse, 2008) and the 2008 BIOPEARL II expedition to the Amundsen Sea in west Antarctica (fig. 1, table 1), a large number of polychaete worms were collected (Linse et al., 2013; Neal et al., in prep.). Polynoidae were particularly abundant in the Amundsen Sea ($n > 5000$) and were represented by 23 species. Currently there are about 66 recognised polynoid species in the Southern Ocean (WoRMS, 2013), and hence the Amundsen Sea collection represents a reasonable coverage of the polynoid diversity of this region. A large number (>2000) of these individuals were small polynoids either considered to be indeterminable juveniles or to be representatives of the small-sized species *Herdmanella gracilis* (based on locality we consider these *Herdmanella gracilis* Ehlers sensu Augener, 1929, rather than Ehlers, 1908). As specimens were preserved both for molecular studies in ethanol and for morphological studies in formaldehyde, there was an opportunity to use molecular taxonomy methods to analyse the validity of the species determination of *H. gracilis*, to examine the genetic heterogeneity in populations, and to commence investigation of the ecological significance of this abundance of juvenile Polynoidae.

Methods

Field methods

The macrobenthic samples were collected by epibenthic sledge (EBS) during the BIOPEARL I and II expeditions with RRS *James Clark Ross* (JR144 and JR179) (fig. 1, table 1). The EBS (for a detailed description see Brenke (2005)) consists of an epi- (lower) and a supra- (upper) net, each with an opening of 100-cm width and 33-cm height, 500-μm mesh size on the sides and ending in cod ends with a mesh size of 300 μm. The EBS was hauled over the seabed at 1 knot for 10 min. On deck, the samples of the first 1000-m and 1500-m and the first two 500-m EBS hauls per station were immediately fixed in 96% pre-cooled ethanol and kept for 48 h in −20°C for later DNA extraction, while the samples of the remaining 500-m, 1000-m and 1500-m EBS hauls per station were fixed in 4% formaldehyde for morphological analysis.

Morphological investigation

Where possible, live specimens were examined aboard ship, with sorted samples being preserved individually and images taken on ship with a Nikon Coolpix camera mounted on a Leica stereo microscope. In the laboratory, Leica MZ6 and DM5000 stereo and compound microscopes were used to further identify polynoid specimens. Images of these specimens were captured using a Zeiss V.20 and AxioCam HRc, and a Leica DFC 480 dedicated camera system connected to the DM5000.

Molecular work

In total, DNA was extracted from 33 ingroup specimens. Eleven specimens morphologically agreed with *Herdmanella gracilis* Ehlers sensu Augener, 1929, 19 with *Austrolaenilla antarctica*, three with *A. pelagica* (table 2). Eight outgroup sequences were included (table 2.) Five outgroup sequences were obtained from GenBank and three were generated as a part of wider polynoid study (Wiklund et al., in prep.). Based on the availability of sequences, we included other species currently in the subfamily Polynoinae (*Harmothoe fuligineum* (Baird, 1865), *Harmothoe oculinarum* (Storm, 1879), *Bylgides groenlandicus* (Malmgren, 1867), *Antarctinoe ferox* (Baird, 1865), *Malmgrenia mcintoshi* (Tebble & Chambers, 1982)), as well representatives of three other subfamilies (*Macellicephala violacea* (Levinsen, 1887), *Eulagisca gigantea* Monro, 1939, and *Lepidasthenia berkeleyae* Pettibone, 1948).

DNA was extracted from parapodia with a DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer.

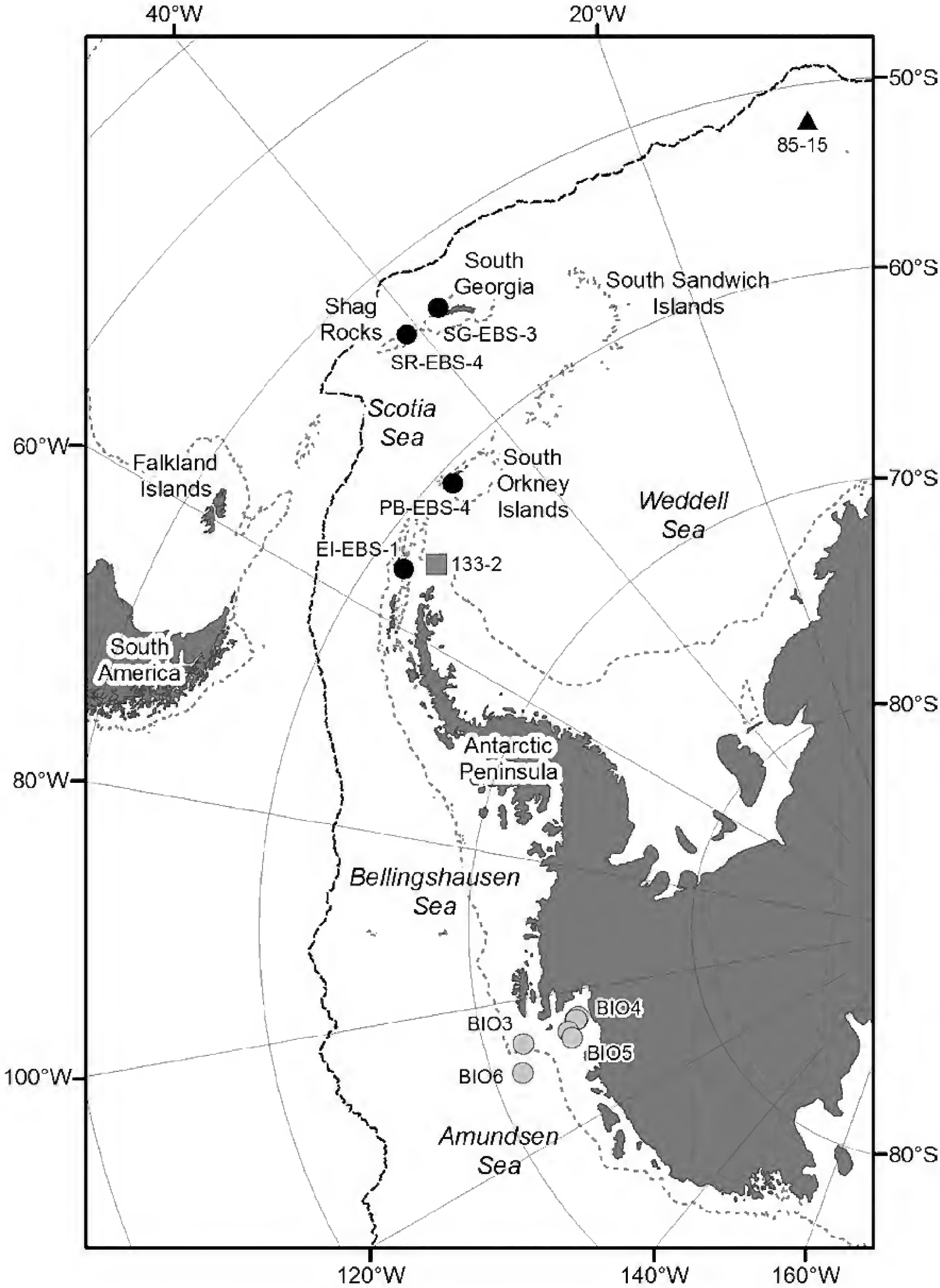

Figure 1. Map showing sampling localities. Black circles refer to BIOPEARL I samples, grey circles to BIOPEARL II samples, grey square to ANDEEP III samples and black triangle to ANDEEP-SYSTCO samples.

Table 1. Details of sampling stations used in this study. Latitude and longitude are provided for the ship's location when the sampling device first landed on the seafloor.

Cruise	Locality	Station	Depth (m)	Latitude	Longitude
ANDEEP III	Weddell Sea (WS)	133	1580	62° 46' 44" S	53° 2' 34" W
ANDEEP - SYSTCO	Weddell Sea (WS)	85-15	2752	52° 0' 28" S	8° 0' 13" W
BIOPEARL I	South Georgia (SG)	SG-EBS-3	500	53° 35' 51" S	37° 54' 11" W
BIOPEARL I	Elephant Island (EI)	EI-EBS-1	1500	61° 36' 43" S	55° 13' 3" W
BIOPEARL I	Powell Basin (PB)	PB-EBS-4	500	60° 49' 18" S	46° 29' 6" W
BIOPEARL I	Shag Rocks (SR)	SR-EBS-4	200	53° 37' 41" S	40° 54' 28" W
BIOPEARL II	Amundsen Sea BIO3	BIO3-EBS-1B	500	71° 47' 29" S	106° 12' 50" W
BIOPEARL II	Amundsen Sea BIO3	BIO3-EBS-1C	500	71° 47' 9" S	106° 12'27" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-AGT-1B	1500	74° 21' 28" S	104° 43' 50" W
BIOPEARL II	Amundsen Sea BIO4	BI04-EBS-3E	500	74° 23' 46" S	104° 45' 28" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-1A	1500	74° 21' 35" S	104° 44' 45" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-2B	500	74° 29' 16" S	104° 19' 58" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-3D	500	74° 23' 27" S	104° 46' 2" W
BIOPEARL II	Amundsen Sea BIO5	BIO5-EBS-2A	1000	73° 52' 49" S	106° 18' 59" W
BIOPEARL II	Amundsen Sea BIO5	BIO5-EBS-3A	500	73° 58' 19" S	107° 25' 22" W
BIOPEARL II	Amundsen Sea BIO6	BIO6-EBS-3D	500	71° 20' 56" S	109° 57' 53" W

Three genes were targeted: the 'barcoding' mitochondrial (mt) protein-coding gene CO1, the mt non-coding 16S and the nuclear (n) protein-coding H3 gene. About 650 bp or 350 bp of CO1, 500 bp of 16S and 300 bp of H3 were amplified using primers listed in table 3. PCR mixtures contained 1 μl of each primer (10μM), 2 μl template DNA and 21 μl Red Taq DNA Polymerase 1.1X MasterMix (VWR) in a mixture totalling 25 μl. The temperature profile was as follows: 96°C for 240 s, followed by (94°C for 30 s, 48°C for 30 s then 72°C/60 s)*35 cycles, followed by 72°C for 480 s. PCR purification was performed using a Millipore Multiscreen 96-well PCR Purification System, and sequencing was performed on an ABI 3730XL DNA Analyser (Applied Biosystems) at the Natural History Museum Sequencing Facility, using the primers mentioned above. Overlapping sequence fragments were merged into consensus sequences using Geneious (Drummond et al., 2007). CO1 and H3 sequences were aligned using MUSCLE (Edgar, 2004) with default settings, while 16S sequences were aligned using MAFFT (Katoh et al., 2002) with default settings, both programs provided as plug-ins in Geneious. The program jModelTest (Posada, 2008) was used to assess the best model for each partition (CO1, 16S and H3) with BIC, which suggested GTR+I+G as the best model for all of the genes. The data was partitioned into the three parts (16S, H3, COI), the evolutionary model mentioned above was applied to each partition and corresponding codon position. The parameters used for the partitions were unlinked. Bayesian phylogenetic analyses (BAs) were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were run three times with the CO1 separate dataset, and with the CO1, H3 and 16S combined dataset, for 10,000,000 generations. Of these, 2,500 000 generations were discarded as burn-in. Haplotype networks using statistical parsimony (Templeton, 1992) were constructed with the program TCS (Clement et al., 2000). In total, 28 CO1 sequences of *Austrolaenilla antarctica* and *Herdmanella gracilis* were cut to the same length of 320 bp. As part of data exploration, different statistical limits ranging from 90% (the lowest limit available in TCS) to 95% were employed. The distances among CO1 sequences were calculated in MEGA version 5.0 (Tamura et al., 2011) and expressed as K2P distance (uncorrected p-distances were also calculated, but the results were very similar) for the purpose of comparison with other studies.

Results

Morphology

During the morphological investigation, a large number of (>2000) small polynoid specimens (fig. 2a and b) were found and at first considered to be indeterminable juveniles (fig. 2c). Upon closer examination, two morphotypes were distinguished: those with cephalic peaks (fig. 3a) and those without cephalic peaks (fig. 3b). No other morphological differences were found between these two morphotypes using light microscopy. The cephalic peaks were not reported in the descriptions either by Ehlers (1908) or Augener (1929); therefore, only specimens without cephalic peaks were assigned to *Herdmanella gracilis*.

Table 2. Taxa studied, outgroups, DNA identification, collection sites, haplotype ID, Clade ID and NCBI GenBank accession numbers. Voucher numbers are available through the GenBank website.

Morphology ID	DNA ID	Locality	Site	Depth (m)	Haplotype #	Clade	COI	16S	H3
Australaenilla antarctica	*A. antarctica*	Amundsen Sea	BIO3-1	500	14	2	KJ676619	n/a	n/a
"	"	"	"	"	19	2	KJ676624	n/a	n/a
"	"	"	BIO4-1	1500	18	2	KJ676623	n/a	n/a
"	"	"	BIO4-3	500	1	3	KJ676612	KJ676606	KJ676637
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	3	3	KJ676613	n/a	n/a
"	"	"	"	"	4	3	KJ676614	n/a	n/a
"	"	"	"	"	5	3	KJ676615	n/a	n/a
"	"	"	"	"	6	3	KJ676616	n/a	n/a
"	"	"	BIO5-2	1000	16	2	KJ676621	KJ676606	KJ676637
"	"	"	BIO5-2	1000	17	2	KJ676622	n/a	n/a
"	"	Elephant Is.	EI-EBS-1	1500	15	2	KJ676620	KJ676606	KJ676637
"	"	South Georgia	SG-EBS-4	500	SG	1	KJ676631	n/a	n/a
"	"	Weddell Sea	138	1580	13	2	KJ676618	n/a	n/a
"	"	"	85-15	2752	13	2	"	n/a	n/a
Herdmanella gracilis	*A. antarctica*	Amundsen Sea	BIO4-3	500	2	3	KJ676625	n/a	n/a
"	"	"	"	"	1	3	KJ676612	KJ676606	KJ676637
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	7	3	KJ676626	n/a	n/a
"	"	"	"	"	8	3	KJ676627	n/a	n/a
"	"	"	"	"	9	3	KJ676617	n/a	n/a
"	"	"	"	"	10	3	KJ676628	n/a	n/a
"	"	"	"	"	11	3	KJ676629	n/a	n/a
"	"	"	"	"	12	3	KJ676630	KJ676606	KJ676637
"	juvenile indet.	Powell Basin	PB-EBS-3	500	n/a	B	KJ676636	KJ676610	KJ676641
"	juvenile indet.	Shag Rocks	SR-EBS-4	500	n/a	B	"	n/a	n/a
Australaenilla pelagica	*A. pelagica*	Amundsen Sea	BIO4-3	500	n/a	A	KJ676632	KJ676607	KJ676638
"	"	"	BIO5-3	500	n/a	A	"	n/a	n/a
"	"	"	"	"	n/a	A	"	n/a	n/a
Antarctinoe ferox	outgroup				n/a	outgroup	KJ676611	n/a	n/a
Bylgides groenlandicus	outgroup	GenBank			n/a	outgroup	HQ024272	n/a	n/a
Eulagisca gigantea	outgroup	Amundsen Sea			n/a	outgroup	KJ676633	KJ676608	KJ676639
Harmothoe fuligineum	outgroup	Amundsen Sea			n/a	outgroup	KJ676634	KJ676609	KJ676640
Harmothoe oculinarum	outgroup	GenBank			n/a	outgroup	AY894314	n/a	n/a
Lepidasthenia berkeleyae	outgroup	GenBank			n/a	outgroup	HM473443	n/a	n/a
Macellicephala violacea	outgroup	GenBank			n/a	outgroup	JX119016	n/a	n/a
Malmgrenia mcintoshi	outgroup	GenBank			n/a	outgroup	JN852935	n/a	n/a

Table 3. List of primers used in this study.

Primer	Sequence 5'–3'	References
16SF	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (2000)
H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (2000)
LCO	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
355R	GGGTAAACTGTTCATCCTGTTCC	Nylander et al. (1999)

Table 4. Within- and between-clade distances as measured by K2P, expressed as mean % (range in brackets).

	Within-clade distance	Between-clade distance					
		A	B	C	C1	C2	C3
A	0	–	–	–	–	–	–
B	0.45 (0.3–0.6)	15.4 (14.3–16)	–	–	–	–	–
C	2.9 (0–7.3)	18.3 (14.8–20.9)	14.4 (12.4–16.3)	–	–	–	–
C1	–	–	–	–	–	–	–
C2	2.5 (0.3–4.1)	–	–	–	6.7 (5.4–7.3)	–	–
C3	1 (0–3.5)	–	–	–	6.4 (5.4–7.1)	4.3 (2.9–5.1)	–

No morphological differences were found among individuals lacking cephalic peaks assigned to *H. gracilis*, and these were therefore assumed to belong to a single species, based on morphology. A short description of the juveniles initially thought to be *H. gracilis* is provided here.

Systematics

Polynoidae Malmgren, 1867

Juvenile, indeterminable

Figures 2, 3.

Material examined. Over 2000 specimens, from BIOPEARL I and II expeditions to the Amundsen Sea, Antarctic, in March 2006 and March 2008, cruise numbers JR144 and 179, station numbers listed in table 1, depth 500 m.

Description. Voucher material. Length excluding palps 1.5 mm, 14–15 segments, 8 pairs of elytra. Colour of preserved specimen white to creamy yellow, in live specimens anterior body translucent, the posterior body bright yellow to orange. Prostomium bilobed, rhomboid to oval, anterior lobes rounded but without cephalic peaks; 2 pairs of small, black, subdermal eyes present, anterior pair positioned medially at widest part of prostomium. 3 antennae; median antenna often missing and only large antennophore present, inserted distally on prostomium, two lateral antennae inserted anteroventrally on prostomium, styles short, slender, papillated. Pair of long (twice length of prostomium), thick, smooth palps present, narrowing distally. Proboscis when extended with 2 pairs of amber-coloured jaws and 9 pairs of small, equal-sized triangular papillae on the rim. Two pairs of tentacular cirri present, lateral to prostomium, styles slender, papillated, tentaculophores of similar size, tentacular segment with notochaetae, few, stout, serrated. Parapodia biramous, notopodia smaller than neuropodia with long, slender, papillated dorsal cirrus; notochaetae present in moderate numbers, stout, straw-like in colour, serrated, much shorter than neurochaetae; neuropodia with long, slender ventral cirrus inserted proximally; neurochaetae numerous, extremely long, thin, almost capillary-like, all unidentate. Elytra often missing, when present small, ovoid, translucent with rough surface, with sparse microtubercules only, some elongated papillae irregularly present on surface and fringe. Pygidium conical, anal cirri not observed.

Remarks. The specimens morphologically agree with the description of *Herdmanella gracilis* Ehlers, 1908; however, it was decided not to assign them to this species without a molecular assessment considering that the specimens are likely to be juveniles, and the type locality (East Africa) is far distant from the Amundsen Sea.

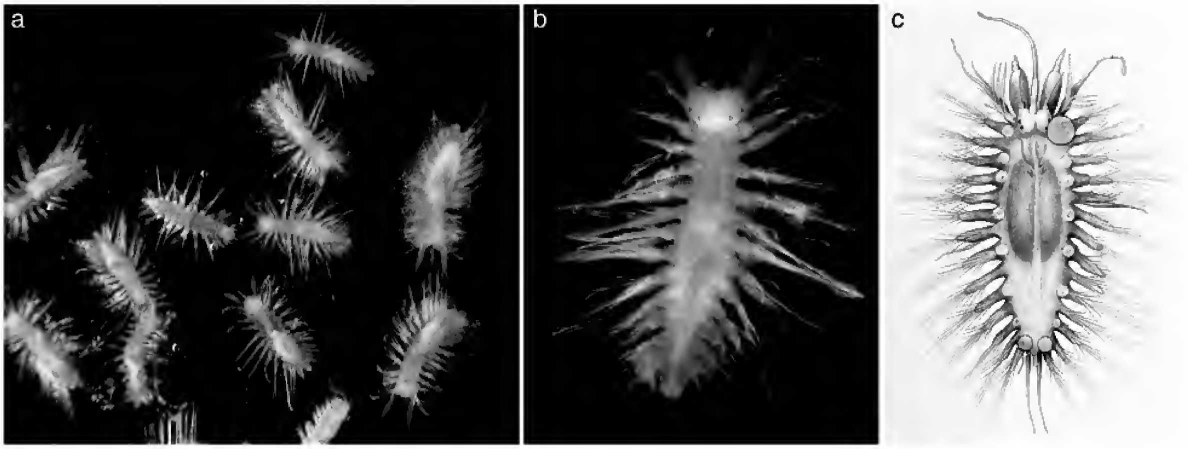

Figure 2. Juvenile Polynoidae: a, image of live specimens agreeing morphologically with *Herdmanella gracilis* Ehlers, 1908; b, detail of specimen; c, drawing of *H. gracilis* from the orginal description published by Ehlers (1908).

Molecular data

The results from the molecular phylogenetic methods based on the CO1 gene only (fig. 4) and on the combined analysis of CO1, 16S and H3 genes (fig. 5) suggest that *Herdmanella gracilis*-like specimens from the Southern Ocean represent juvenile stages of at least two species (clade B and C in fig. 4a). The identity of the juvenile specimens collected in the Powell Basin and at Shag Rocks (clade B in fig. 4a) remains unresolved; however, *Herdmanella gracilis*-like specimens from the Amundsen Sea (clade C) were a close match with adult *Austrolaenilla antarctica* Bergström, 1916, forming a well-supported monophyletic group. The *A. antarctica* clade forms three subclades (C1, C2 and C3), and CO1 diversity is high, with an average K2P distance of 2.9% (range 0–7.3%) (table 4). The changes were found in the third codon position and did not result in changes to amino sequences once translated. Additionally, mt16S and nH3 sequences were obtained for representatives of clades C2 (n = 2) and C3 (n = 3). In 16S, the genetic distance within clade C was reduced to <1% for all specimens, and in nuclear *H3* genes, their sequences were identical.

The 28 specimens in clade C, morphologically assigned to *Austrolaenilla antarctica* and *Herdmanella gracilis* represented 20 haplotypes (table 2). Only seven specimens (five of *A. antarctica* and two of *H. gracilis*) belonged to the same haplotype (no. 1), and all of these were from the Amundsen Sea station BIO4, 500 m depth (table 2). Two specimens from the deep Weddell Sea shared one haplotype, no. 13. The rest of the specimens were all unique haplotypes. A single haplotype network was not recovered using a 90% connectivity limit (11 steps), the lowest limit available in the TCS program. These settings in TCS resulted in the South Georgian haplotype not connecting to the main network formed by all other haplotypes, no. 1–19 (fig. 6a). The same result was obtained using a 91% connectivity limit (results not shown). Ultimately, increasing the parsimony limit to 95% (seven steps) resulted in a breakdown into five haplotype networks (fig. 6b). Three of

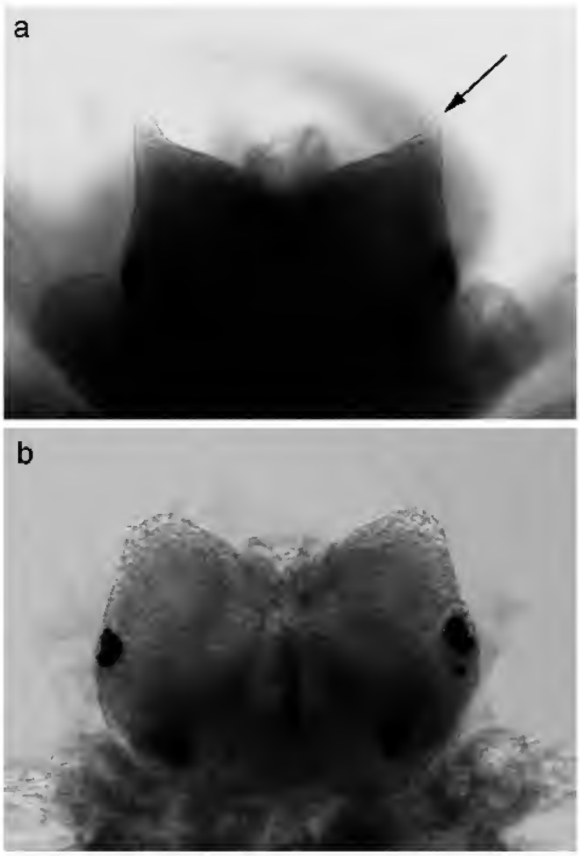

Figure 3. Presence of cephalic peaks in juvenile polynoids: a, type 1 juvenile of *Harmothoe fuligineum*—cephalic peaks clearly present (arrowed); b, type 2 juvenile—cephalic peaks absent, consistent with *H. gracilis* Ehlers, 1908.

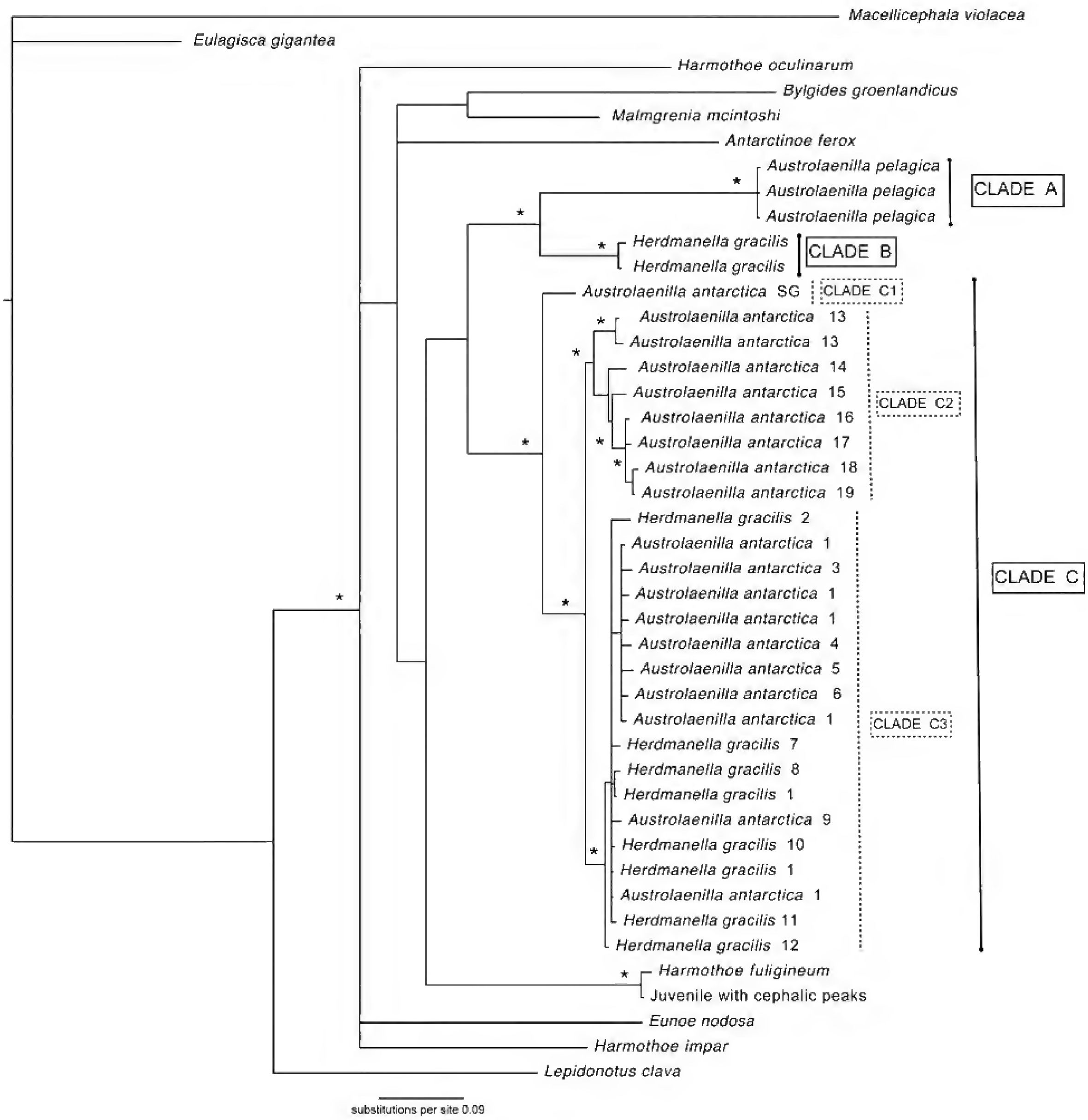

Figure 4. Phylogenetic tree from Bayesian consenus analysis based on CO1 (mtDNA) only. Stars represent significant node values (≥95%) for Bayesian posterior probabilities. Clade numbers and letters refer to table 2 and the main text.

these - South Georgian (SG), Amundsen Sea Station BIO3 (no. 14), and Weddell Sea (no. 13) were represented by single haplotypes only. Four haplotypes from various sampling stations and depths in the Amundsen Sea (nos 16–19) formed a separate network. The largest network was composed of haplotypes of *A. antarctica* and *H. gracilis* from the Amundsen Sea station BIO4, 500 m (haplotypes no. 1 -12) with the addition of a single haplotype from Elephant Island (haplotype no. 15).

Discussion

Taxonomy and genetic diversity: Herdmanella gracilis *in the Southern Ocean*

Ever since Augener (1929) first identified small polynoid specimens from the Southern Ocean as *Herdmanella gracilis* Ehlers, 1908, this species was considered to have an Antarctic, as well as Indian Ocean (type locality) distribution. However, given

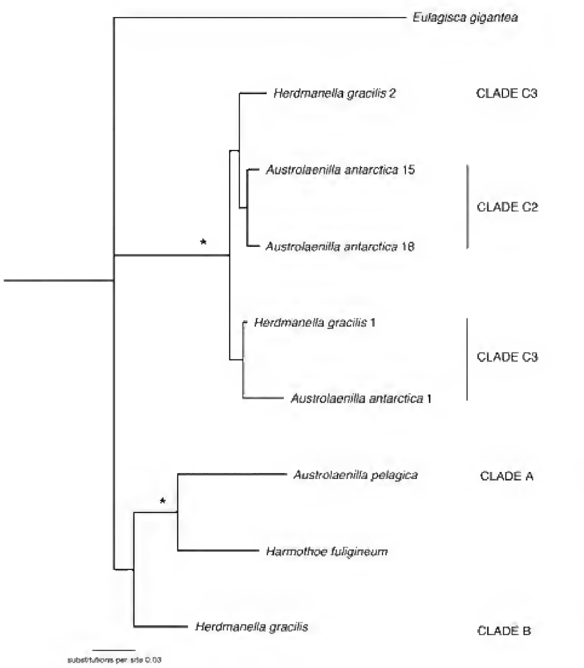

Figure 5. Phylogenetic tree from Bayesian consenus analysis based on the CO1, 16S (mtDNA) and *H3* (nDNA) combined dataset of selected specimens. Stars represent significant node values (≥95%) for Bayesian posterior probabilities.

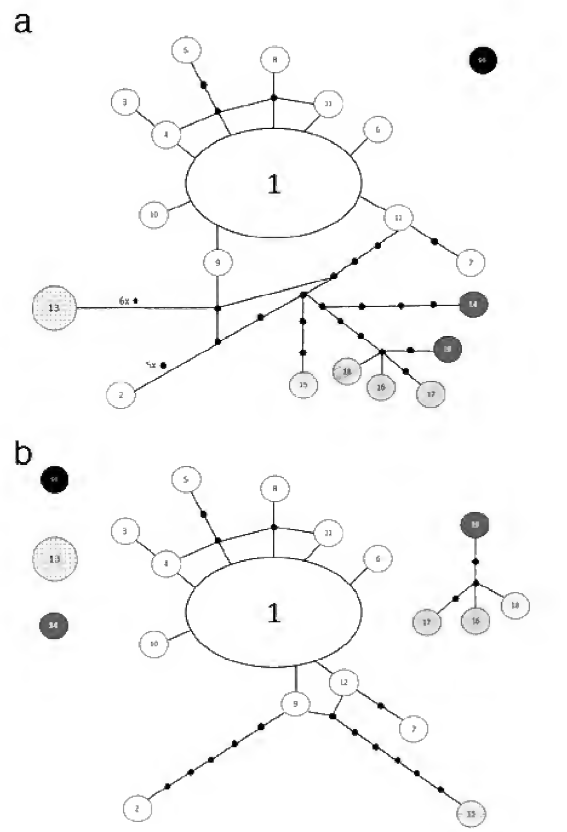

Figure 6. Haplotype network of 20 haplotypes based on *Austrolaenilla antarctica* and *Herdmanella gracilis* mtCO1 data: a, representing 91% connection limit; b, representing 95% limit. Small black circles represent unsampled haplotypes, large circles represent the sampled haplotypes with their size proportional to the frequency of the haplotype (n = 1, 2 and 7). Numbers in the shapes correspond to haplotype identification numbers (see table 2). Different geographical locations are coded—white circles are Amundsen Sea BIO4, 500-m depth; light grey circles are BIO4, 1500-m depth; dark grey circles are BIO3; cross-hatched circles are Weddell Sea; horizontal hatched circles are Elephant Island; and black circles are South Georgia.

the small size of the specimens, the longstanding suggestion by many authors was that they were juveniles, with links suggested to the genus *Austrolaenilla* (*Antinoe* in Monro, 1930) or *Harmothoe* Kinberg, 1855 (Augener, 1929; Hartmann-Schröder, 1974; Pettibone, 1976. During the morphological investigation of a large number of these small polynoids in our study, two morphotypes were distinguished: those with cephalic peaks (a feature not reported in *Herdmanella gracilis*) (fig. 3a) and without cephalic peaks (fig. 3b) (consistent with *H. gracilis*). The small specimens with cephalic peaks are not the subject of this paper, but molecular methods employed in a wider phylogenetic study of Southern Ocean Polynoidae have identified these as juveniles of *Harmothoe fuligineum* (Baird, 1865) (Wiklund et al., in prep.). However, when identifiers are presented with a large number of these small worms (thousands in this study), the two morphotypes can be confused, as the cephalic peaks can be easily overlooked.

The molecular methods based on the CO1 gene only and on combined analysis of the CO1, 16S and H3 genes suggest that *Herdmanella gracilis*-like specimens from the Southern Ocean, which are morphologically indistinguishable, do in fact represent juvenile stages of at least two species (clades B and C in fig. 4). It is very likely that greater sequencing effort would identify other species within the *Herdmanella gracilis*-like juveniles. The identity of the adult stage for *H. gracilis*-like juveniles from the Scotia Sea (clade B in fig. 4) remains unresolved. *Herdmanella gracilis*-like specimens from the Amundsen Sea are a close match with the adult stage of *Austrolaenilla antarctica* Bergström, 1916, a common species with wide circumpolar distribution, with *Austrolaenilla antarctica* and *H. gracilis* constituting a well-supported monophyletic group (clade C in fig. 4). In CO1, the genetic diversity in clade C was rather high (see further discussion below); the smallest distance between *A. antarctica* and *H. gracilis*-like specimens was 0%, but the average distance was approximately 4%. However, the gap between 'good' species, e.g. between *A. antarctica* and *A. pelagica* was on average 18.3 (range 14.8–20.9) (table 4), which suggests that an average of 4% distance may constitute potential intraspecific variation. Given this relatively large 'barcoding gap', even for specimens sampled from the same locality, 16S and H3 sequences were obtained for

a small number of representative specimens with high CO1 divergences. In 16S, the genetic distance was reduced to <1%, and in nuclear H3 genes, these sequences were identical.

Additionally, haplotype networks with a 95% connectivity limit provide a useful tool for species delimitation (e.g. Hart and Sunday, 2007; Monaghan et al., 2006). For the purposes of clarifying the identity of the *Herdmanella gracilis*-like specimens from the Southern Ocean, the haplotype networks confirmed the identity of many of these specimens as juveniles of *Austrolaenilla antarctica*, even under the strict 95% connectivity limit (fig. 6b, table 2). However, the status of specimens morphologically agreeing with *A. antarctica* (from various locations in the Southern Ocean) as a single species can be questioned. The single specimen from South Georgia (corresponding to clade C1 in fig. 4) could be justified as a different species since the single haplotype network could not be recovered, even under a 90% limit (fig. 6a). Specimens from clades C2 and C3 formed a single haplotype network under 90 and 91% limits (fig. 6a), but failed under the more conservative 95% limit (fig. 6b). However, the network of the haplotype numbers 16–19 fails to be connected to the main network by a single step.

As with the 'barcoding gap', the coveted 95% connectivity limit may not work well for *Austrolaenilla antarctica*, although the presence of cryptic species cannot be discounted. A larger sampling effort, covering other locations and including a greater number of specimens of *A. antarctica*, would be needed to clarify its status as a single or cryptic species. Several studies using DNA to delimit Southern Ocean taxa based on mitochondrial sequence data suggested the discovery of new (often cryptic) species, e.g. the polychaete *Glycera kerguelensis* (Schüller, 2010), the pycnogonids *Nymphon australe* (Mahon et al., 2008) and *Colossendeis megalonyx* (Krabbe et al., 2010), amphipods (Loerz et al., 2009; Baird et al., 2011), nudibranch *Doris kerguelensis* (Wilson et al., 2009), crinoid *Promachocrinus kerguelensis* (Wilson et al., 2007), ostracods (Brandão et al., 2010) or octopus (Allcock et al., 2011), while fewer studies supported a true circumpolar distribution (e.g. Raupach et al., 2010; Arango et al., 2011). A summary of the extent of the barcoding studies in the Southern Ocean has been provided by Grant et al. (2011) and Allcock and Strugnell (2012). Nygren (2013) provides an in depth review of cryptic diversity in polychaetes.

The problem of species delimitation and species concepts has a long history in biology (e.g. de Queiroz, 2007; Wiens, 2007; Wilkins, 2011). In recent years, the molecular approach has been added to the toolbox for both species identification and species delimitation. As already mentioned above, a common approach, which we also adopted here, is to search for discontinuities in DNA sequence variation either through the statistical parsimony method (Templeton et al., 1992), which is used to build haplotype networks, or by the discovery of a barcoding gap (Hebert et al., 2003). This gap is supposed to represent the difference between the highest genetic distance found within species and the lowest genetic distance found between species. The most common part of DNA used in animal barcoding studies is the CO1 gene of mtDNA. However, this choice of marker, as well as the concept of the barcoding gap itself, has been subject to fierce criticism ever since it was proposed by Hebert et al. (2003). In their review of mitochondrial DNA, Galtier et al. (2009) concluded that, given the heterogeneous evolutionary rate of mtDNA and processes such as hybrid introgression or balancing selection, the universal distance-based 'gaps' for delineation of taxa into species do not exist. This view has been supported by many other workers, adding problems of small sample sizes or narrow geographical coverage, which may affect the size of the 'gap', resulting in extensive literature concerning the use of mtDNA barcodes (e.g. Moritz and Cicero, 2004; Meyer and Paulay, 2005; Meyer et al., 2008; Bergsten et al., 2012; Collins and Cruickshank, 2012). The increasingly accepted approach to species delimitation will rely on examination of mtDNA sequence distance, variation at multiple (including nuclear) genetic loci in a phylogenetic context, careful morphological examination, as well as ecological and biological observations (see references in Nygren, 2013). However, to obtain all these lines of evidence is not always possible and certainly takes time.

The most comprehensive work on barcoding of polychaetes to date was completed by Carr et al. (2011) on Arctic polychaetes. Their analysis of 1876 specimens, representing 333 provisional species, revealed 40 times more between-species sequence divergence in CO1 as opposed to within species (16.5% versus 0.38%), as estimated by Kimura 2 parameter (K2P) distance measure. In Carr et al. (2011), the CO1 barcodes have high discriminatory power for polychaetes because the average observed within-clade divergence in their study was 3.8% (highest within-species divergence was 5.7%), indicating that barcodes naturally form tight clades with low variation. A smaller regional barcoding study on Chilean polychaetes conducted by Maturana et al. (2011) corroborated the results of Carr et al. (2011) by finding that mean pairwise sequence distance comparisons, based on K2P within-species, ranged from 0.2 to 0.4%, while interspecific comparisons were much higher and ranged between 18 and 47%.

Results from our study approach the interspecific differences observed in other research on polychaetes (e.g. Schüller, 2010; Maturana et al., 2011; Carr et al., 2011), with the average K2P distance in CO1 found to range from 14.4 to 18.3% (table 4). However, variable results were obtained for within-species diversity in CO1. There was no divergence in *Austrolaenilla pelagica* clade A (fig. 4) in specimens from various sampling sites in the Amundsen Sea (greatest distance ca. 500 km apart). Similarly, the two juveniles of unknown identity forming clade B (fig. 4a) were separated by less than 1% in CO1, despite the fact that these specimens came from the geographically distant sites of the Powell Basin and Shag Rocks. Additionally, unpublished work by Ramon (pers. comm.) on Antarctic marine larvae revealed a close match to the unknown species in clade B in CO1 and 16S sequences with unidentified polynoid larvae from the Ross Sea. The low level of divergence within these clades (A and B), which also correspond to morphological species, is in agreement with the results of other studies mentioned above. Large distribution areas have often been accepted for marine fauna in the past, but this assumption has been questioned (e.g. Hellberg, 2009). Further, the discovery of cryptic species also challenges this idea (Nygren, 2013). However, wide distributions have been

shown for polychaetes by Schüller and Hutchings (2012), who demonstrated long-distance dispersal in *Terebellides gingko* using 16S rDNA sequences.

In contrast, a very high level of diversity was observed in the *Austrolaenilla antarctica* clade A, with the greatest distance being 7.3%. The specimens in the *A. antarctica* clade were collected from several locations in the Southern Ocean (table 1), covering a total geographical distance of ca. 6000 km (if following the shelf of islands in the Scotia Arc). Reflecting this, the *A. antarctica* clade itself is formed of three clades (C1, C2 and C3). The most divergent specimen from South Georgia (clade C1) may possibly represent a cryptic species, a notion also supported by the haplotype network analysis. Clade C2 is comprised entirely of adults that agreed morphologically with *A. antarctica*, with an average distance of approximately 2.5% (range 0.03–4.1% (table 4)). These specimens were mostly collected at locations from across the Amundsen Sea at varying depths, but the clade also contains one specimen from Elephant Island, 1500 m (BIOPEARL I collection), and the most divergent specimen is from the abyssal Weddell Sea (ANDEEP collection). Clade C3 includes exclusively juveniles and adults collected from a single sampling station on inner shelf BIO4 at 500 m. Individuals from a single station were selected to establish the identity of the juveniles previously identified as *Herdmanella gracilis* in order to reduce the diversity introduced by factors such as geographical or bathymetrical distance. Even though the average distance was low at 1% within this clade, the highest CO1 distance was 3.5% (table 4). In this study, only a small number of individuals were sequenced from >1000 specimens collected at this particular site, and potential future work specifically aimed at population genetics may provide further insights.

In a large-scale study of lumbricid earthworms in Britain, King et al. (2008) found that two morphs of *Allolobophora chlorotica* (with over 14% divergence at CO1) are interbreeding and therefore represent a single taxon. This conclusion was further supported by amplified fragment length polymorphism (AFLP) markers. In their overall survey of CO1 sequence diversity of nine species of British earthworms, represented by 71 individuals, they found that sequence divergences within species varied from 0.35% to 12.35%, highlighting yet again the lack of a universal threshold for the barcoding gap, even within closely related species, which is similar to our results for two recognised and one suspected species of *Austrolaenilla*. Similar results were obtained in a recent study by Achurra and Erséus (2013) examining population structure of the aquatic oligochaete *Stylodrilus heringianus* Claparède, 1862, covering its range on a European scale (Estonia to Spain) using sequences of the mt CO1 and two nuclear genes: internal transcribed spacer region and histone 3. The authors also found a large CO1 diversity, with a maximum distance of 7.7%, as measured by K2P. Nevertheless, nuclear genes failed to confirm any lineage separation, and it was concluded that the sampled specimens all belong to the same species, asserting that the mitochondrial single-locus approach can be problematic for the accurate delimitation of species.

Of several hypotheses proposed to explain high diversity in mitochondrial sequence data and its discordance with nuclear genes (see e.g. King et al., 2008), that of incomplete speciation following isolation in distinct glacial refugia is of particular relevance to the Southern Ocean fauna. The Earth's climatic history is marked by alternating glacial and interglacial periods. During the ice ages, populations of plants and animals have shown primarily two survival strategies: migration or survival *in situ*, helped by the existence of glacial-free refugia. Populations in different refugia will diverge from one another through genetic drift, which may lead to reproductive isolation of those populations. The recent insights into the history of glaciation in Antarctica have shown that at glacial maxima, grounded ice sheets extended over much of the Antarctic continental shelf (Thatje et al., 2005). As a result, most (if not all) available habitat for marine benthos was destroyed, making this group particularly vulnerable to extinction. Earlier workers such as Dell (1972) proposed that the continental shelf fauna was completely eradicated by glacial cycles and recolonised from the deep sea. Others suggested that some fauna survived on the continental shelf itself in ice-free refugia (Clarke et al., 2004). These ice-free regions existed on a range of temporal and spatial scales, and not all areas around the continent have been glaciated at the same time (Anderson et al., 2002). As such, isolation of historic populations in Cenozoic glacial refugia could provide some explanation for the high mtDNA diversity in our modern Antarctic polychaete populations. The shelf of the Amundsen Sea, the site of collection of most specimens used in this study, is particularly complex in its bathymetry as a result of past as well as present day glacial activity (Lowe and Anderson, 2002).

Status of the genus Herdmanella

Darboux, 1899, erected the genus *Herdmanella* for the species *Polynoe* (?) *ascidioides* McIntosh, 1885, which was found at station 160 of the Challenger Expedition in the Southern Ocean (42°42'S 134°10'E, south of Australia) in the branchial chamber of an ascidian on red clay at 4755m depth. This is therefore the type species of the genus by monotypy. McIntosh, 1885, himself mentioned some similarity between his species *Polynoe* (?) *ascidioides* and *Polynoe (Macellicephala) mirabilis* McIntosh, 1885. Uschakov (1971) also compared this species to the genus *Macellicephala*. However, Hartmann-Schröder (1974) referred to it as *Macellicephala (Macellicephala) ascidioides* (McIntosh, 1885), saying that it is incompletely known, but explicitly making *Herdmanella* a junior synonym of *Macellicephala* McIntosh, 1885. More recently, Pettibone (1976) referred to *Polynoe* (?) *ascidioides* as a 'doubtful Polynoidae'. The holotype is apparently the only specimen of this species that has been reported. This holotype has been re-examined by Muir (1982), who found it to be in bad condition and lacking a head, so it cannot with certainty be referred to any polynoid subfamily. It is clear, therefore, that *Polynoe* (?) *ascidioides* McIntosh, 1885 must be regarded as a *nomen dubium* (a name of unknown or doubtful application). If the name of the type species of a genus (*Polynoe* (?) *ascidioides*) is a *nomen dubium*, it follows that the generic name *Herdmanella* must also be a *nomen dubium*. It is not clear why previous authors did not arrive at this conclusion.

Although our study provides clear evidence that specimens from the Southern Ocean morphologically consistent with

Herdmanella gracilis are in fact juveniles of at least two species in the polynoid genus *Austrolaenilla*, we cannot come to a definite conclusion about the identity of *H. gracilis* from the type locality in the equatorial Indian Ocean. We have, however, certainly strengthened the longstanding suggestion that it is a juvenile, and the results from the Southern Ocean point towards the genus *Austrolaenilla* as adult counterparts, but no adults of *Austrolaenilla* species are known from the equatorial Indian Ocean. Similarly *Herdmanella aequatorialis* from the Gulf of Guinea, currently regarded as the other valid species in the genus *Herdmanella*, is also likely to be a juvenile of an, as yet unknown, polynoid. *Austrolaenilla meteorae* (originally placed in *Harmothoe*) was described by Hartmann-Schröder (1982) from the equatorial Atlantic Ocean off West Africa and may possibly be the adult of *H. aequatorialis*. As the genus *Herdmanella* is now regarded as a *nomen dubium*, the two species *H. gracilis* and *H. aequatorialis* are now without a generic placement, and as they probably represent juveniles of other species they are also best regarded as *nomina dubia*.

Ecology of polynoid juveniles

Larval ecology is important to understanding patterns and processes influencing marine populations, communities and ecosystems. However, one of the limitations to the study of community ecology is the ability to correctly identify marine larvae and juveniles. As this study shows, not only is it problematic to attempt to distinguish juveniles of related polynoid species on the basis of morphology alone, but juveniles may well have been considered different species in the past. This of course will have repercussions for subsequent analysis of community structure. It is indeed rather arbitrary how to treat a large collection of juveniles in an ecological analysis if their identity is spurious. Without being able to identify these correctly, it may be sensible to exclude indeterminable juveniles from the analysis. However, the distribution and abundance patterns of juveniles are of great interest, given that very little is known about this subject.

The large number of juveniles (>2000) belonging to several polynoid species collected in the Amundsen Sea is suggestive of recent spawning. The samples were collected towards the end of the austral summer in early March of 2008. This points to a potentially synchronised summer spawning event of at least two polynoid species—*Austrolaenilla antarctica* and *Harmothoe fuligineum*. The largest numbers were found at 500 m depth (the shallowest horizon sampled during the BIOPEARL II cruise), and they were exceptionally abundant at two inner-shelf stations in Pine Island Bay. Although recent studies indicated the existence of so-called food banks available to benthos throughout the year (Smith et al., 2002; Glover et al., 2008), it is likely that recruitment predominantly occurs during the summer months and is linked to high food availability. In addition, the inner-shelf BIOPEARL II stations are characterised by the presence of polynyas, areas known for high productivity (Arrigo and Van Dijken, 2003). The high number of polynoid juveniles in this region is likely linked to this. The analysis of biodiversity and ecology of polychaetes from Amundsen Sea is currently underway (Neal et al., in prep.).

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Original specimens and type localities of early described polychaete species (Annelida) from Norway, with particular attention to species described by O.F. Müller and M. Sars

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Abstract

Oug, E., Bakken, T. and Kongsrud, J.A. 2014. Original specimens and type localities of early described polychaete species (Annelida) from Norway, with particular attention to species described by O.F. Müller and M. Sars. *Memoirs of Museum Victoria* 71: 217–236.

Early descriptions of species from Norwegian waters are reviewed, with a focus on the basic requirements for re-assessing their characteristics, in particular, by clarifying the status of the original material and locating sampling sites. A large number of polychaete species from the North Atlantic were described in the early period of zoological studies in the 18th and 19th centuries. The descriptions were often short or referred solely to general characteristics, which by today's standards are considered inadequate for species discrimination. As a result, a number of taxa among the so-called 'well-known and widely distributed' species have later been confused with morphologically similar species. Close to 100 presently valid species were described from Norwegian waters before 1900. The most prolific contributions were made by O.F. Müller (with about 20 species from 1771–1776) and Michael Sars (with more than 50 species from 1829–1872). Other authors in the 19th century included Anders Ørsted, Heinrich Rathke and Gerhard Armauer Hansen. Descriptions were mostly in Latin (O.F. Müller) or in Norwegian or Danish with the diagnosis in Latin (M. Sars and contemporary naturalists). Original material from O.F. Müller is not known to exist. Original material from M. Sars and contemporary scientists does still exist, but is often not identified as original ('syntypes') and is occasionally spread over several museum collections. Locating original sampling localities ('type localities') has been achieved by combining information from various literature sources, labels of original material (when extant), and knowledge of historic place names.

Keywords Polychaeta, early-described species, original material, sampling sites, Norway

Introduction

The Nordic countries were central in the early studies of marine fauna and flora in scientific history. In the second half of the 18th century, several scientists, e.g. Johan Ernst Gunnerus, Otto Friderich Müller and Otto Fabricius, corresponded with Carl Linnaeus and contributed to his *Systema naturae*, as well as describing new species in their own publications (Anker, 1950; Wolff, 1994; Moen, 2006). In the 19th century, a large number of species were described from Nordic waters by Michael Sars, Anders Ørsted, Heinrich Rathke, Gerhard Armauer Hansen, Anders Johan Malmgren, Henrik Nikolai Krøyer and Ivar Arwidsson, for example. Typically, many of the species are among the most common and abundant in the areas in which they were described.

A number of the early-described species are insufficiently characterised with regard to present-day requirements in species taxonomy. In numerous cases, species have been confused with morphologically similar species and reported from wide geographic areas. From the late part of the 19th century, there emerged a tradition of lumping polychaete species (Barroso et al., 2010). Fauvel (1959) expressed explicitly a view that polychaete species had a high degree of morphological variation and consequently had a wide geographic distribution. It is presently agreed that the reported wide distribution results from confusing similar species with separate distributions and also different responses to environmental conditions. This has been clear for some time from critical morphological studies (e.g. Williams, 1984;

Mackie and Pleijel, 1995; Koh et al., 2003). Furthermore, recent studies have shown that even in more restricted areas, several morphologically similar but genetically different forms have been demonstrated among common species (e.g. Breton et al., 2003; Nygren et al., 2005; Bleidorn et al., 2006).

In Norway, work has been initiated to trace original material and type localities for early-described species of polychaetes. The main intention is to clarify the status of the species and through this establish a basis for characterisation of species in accordance with present-day standards of taxonomy. The advent of molecular genetic methods presents new challenges in taxonomy, while providing powerful tools to discriminate between confused species. It has long been understood that the knowledge of polychaetes in Norwegian waters is incomplete due to many unresolved systematic problems, particularly among early-described species. Close to 100 presently valid species of polychaetes were described from Norwegian waters during the 18th and 19th centuries. The present paper gives a general overview of the early studies, places of collection, nature of original publications and status of original material. The most influential individuals in the 18th and 19th centuries were Otto Friderich Müller and Michael Sars, respectively, and most of the focus is on their contributions. Part of the present work has been carried out under the framework of the Norwegian Taxonomy Initiative, which is a broad-scale program aimed at mapping species diversity in Norway.

Abbreviations

NHMO Natural History Museum, Oslo

NMWC National Museum of Wales, Cardiff

NNHE Norwegian North-Atlantic Expedition

NTNU-VM Norwegian University of Science and Technology, University Museum, Trondheim

USNM National Museum of Natural History, Washington DC

ZMBI Zoological Museum, Berlin

ZMBN University Museum of Bergen

The need to reassess the characteristics of early-described species

The proper characterisation of early-described species is necessary to resolve complexes of confused species and for discriminating and diagnosing related new species. Without this clarification, species descriptions may confuse characters from similar species. The need for precise species identification is crucial in monitoring and for environmental assessment studies, e.g. the European Water Framework Directive, where the detection of species changes is the very basis for assessing to what degree human influences or climate changes are affecting natural ecosystems. Inaccurate species discrimination reduces the sensitivity of monitoring tools.

There is also a need to clarify which of several species is the originally named species when species complexes are resolved. The rapidly expanding use of molecular genetic methods has demonstrated how cryptic species are common in the marine environment (Knowlton, 2000). From Nordic waters, several examples of cryptic species among early-described phyllodocids have been demonstrated (Nygren et al., 2009, 2010; Nygren and Pleijel, 2011). For the nereidid *Hediste diversicolor* (O.F Müller, 1776) and the orbiniid *Scoloplos armiger* (O.F. Müller, 1776), clear genetic differences between populations have been documented (Breton et al., 2003; Bleidorn et al., 2006; Audzijonyte et al., 2008). Furthermore, in international gene sequencing databases such as the database holding DNA-barcoding sequences, BOLD (Barcode of Life Data System) (Ratnasingham and Hebert, 2007), there are several examples of different molecular sequences being uploaded for the same taxon, reflecting the improper discrimination of related species. For example, recent searches in the BOLD database for *H. diversicolor* and *Cirratulus cirratus* (O.F. Müller, 1776) showed three and four putative species, respectively, indicated by DNA barcoding (access date 3 April 2014). The rapidly expanding use of modern genetic analytical techniques, hence, necessitates that correct genetic information can be obtained for early-described species.

In order to clarify the characters of insufficiently described species, the established practice in taxonomy is to examine the original material (type specimens), or in cases where new material is needed, to collect at the same location where the original material was collected (type locality). These specifications imply that the status of the original material should be known, and the locality for collecting new material (type locality) should be fixed. The International Code of Zoological Nomenclature (ICZN) provides rules governing what constitutes original material and how type localities should be fixed (ICZN, 1999). New material may be collected in cases where the original material has been lost, for critical morphological studies that cannot be performed on original material, and for molecular genetic analyses. Material from type localities (topotypic material) may also be of great help if the original specimens are of poor quality but still in a condition to confirm conspecific status. Genetic sequences from the same samples will provide genetic characterisation of the species in question and provide museum vouchers for specimens used in genetic analyses (Pleijel et al., 2008).

The collection of new material is particularly important for genetic characterisation. Attempts to obtain genetic information from old museum specimens have generally failed. Museum specimens have traditionally been preserved in formalin, which degrades and fragments DNA, and may cause a number of changes to the DNA (Skage and Schander, 2007). Protocols have been tested to accommodate the challenge to extract DNA suitable for sequencing without much success (Schander and Halanych, 2003; Skage and Schander, 2007). The general need for new material in genetically supported taxonomic work underlines the importance of critically selecting the place to sample the material for linking molecular genetics to traditional taxonomy. The type locality can provide a link between modern genetically based taxonomy and traditional morphology-based taxonomy.

Table 1. Summary of valid species named by O.F. Müller. Access number and annotations in 'prodromus' (Müller, 1776) is shown: +, species indicated as found and diagnosed by Müller himself; #, species described by other authors; –, no particular indication. Species described in Zoologia Danica are shown by volume number and locality when stated. See Figure 6 for localities.

Valid name	Prodromus: number/reference	Zoologia Danica	Locality (-ies)	Descriptions/revisions
Originally in *Lumbricus*				
Nephtys ciliata	2607/–	Vol. III	Norway (no precise locality)	Fauchald (1963), Rainer (1991)
Cirratulus cirratus	2608/#	–		
Scoloplos armiger	2610/+	Vol. I	Kristiansand	
Scoletoma fragilis	2611/+	Vol. I	Drøbak in Oslofjord	Frame (1992)
Originally in *Amphitrite*				
Amphitrite cirrata	2617/#	–		Müller (1771)
Pista cristata	2620/+	Vol. II	Kristiansand	
Pherusa plumosa	2621/#	Vol. III	Greenland; Norway (no precise locality)	Fabricius (1780); emended J.C. Abilgaard (Haase, 1915)
Pectinaria auricoma	2622/–	Vol. I	Drøbak and Kristiansand	
Originally in *Nereis*				
Hediste diversicolor	2624/#	–		
Hyalinoecia tubicola	2625/+	Vol. I	Drøbak in Oslofjord	
Syllis armillaris	2626/+	–		Müller (1771), Licher (1999)
Eunice pennata	2630/+	Vol. I	Drøbak in Oslofjord	Winsnes (1989), Fauchald (1992)
Nereimyra punctata	2633/+	Vol. II	Drøbak in Oslofjord	Pleijel et al. (2012)
Glycera alba	2634/+	Vol. II	Norway (no precise locality)	
Procerea prismatica	2637/–	–		Nygren (2004)
Spio filicornis	2640/#	–		Fabricius (1780), Meissner et al. (2011)
Originally in *Aphrodita*				
Pholoe longa	2646/#	–		Fabricius (1780), Pettibone (1992)
Originally in *Dentalium* (Mollusca)				
Ditrupa arietina	2853/+	–		ten Hove and Smith (1990)
Orig in *Tubularia* (Cnidaria part)				
Fabricia stellaris	3065/+	–		Müller (1774), Fitzhugh (1990)
Not in 'prodromus'				
Myrianida prolifera (as *Nereis prolifera*)		Vol. II	Norway (no precise locality)	Nygren (2004)
Scololepis squamata (as *Lumbricus squamatus*)		Vol. IV	Helgoland	Most probably described by J.C. Abildgaard

The earliest described species: O.F. Müller and Zoologia Danica

Otto Friderich Müller (1730–1784) (variant spelling Otto Friedrich) was one of the most important early naturalists and one of the pioneers in marine biology (fig. 1). He was Danish and performed most of his studies in Denmark, but came to work in Norway during the 1770s through marriage to a wealthy Norwegian widow. In Norway, he was based in Drøbak, a small settlement about 30 km south of Oslo (at the time called Christiania), but during summer periods he made travels to the south coast of Norway and Norwegian inland areas to collect animals and plants. He described species from a variety of species groups from fresh water as well as marine habitats. In addition to polychaetes, he described species of molluscs, crustaceans, echinoderms and several parasite groups (Anker, 1950; Wolff, 1994).

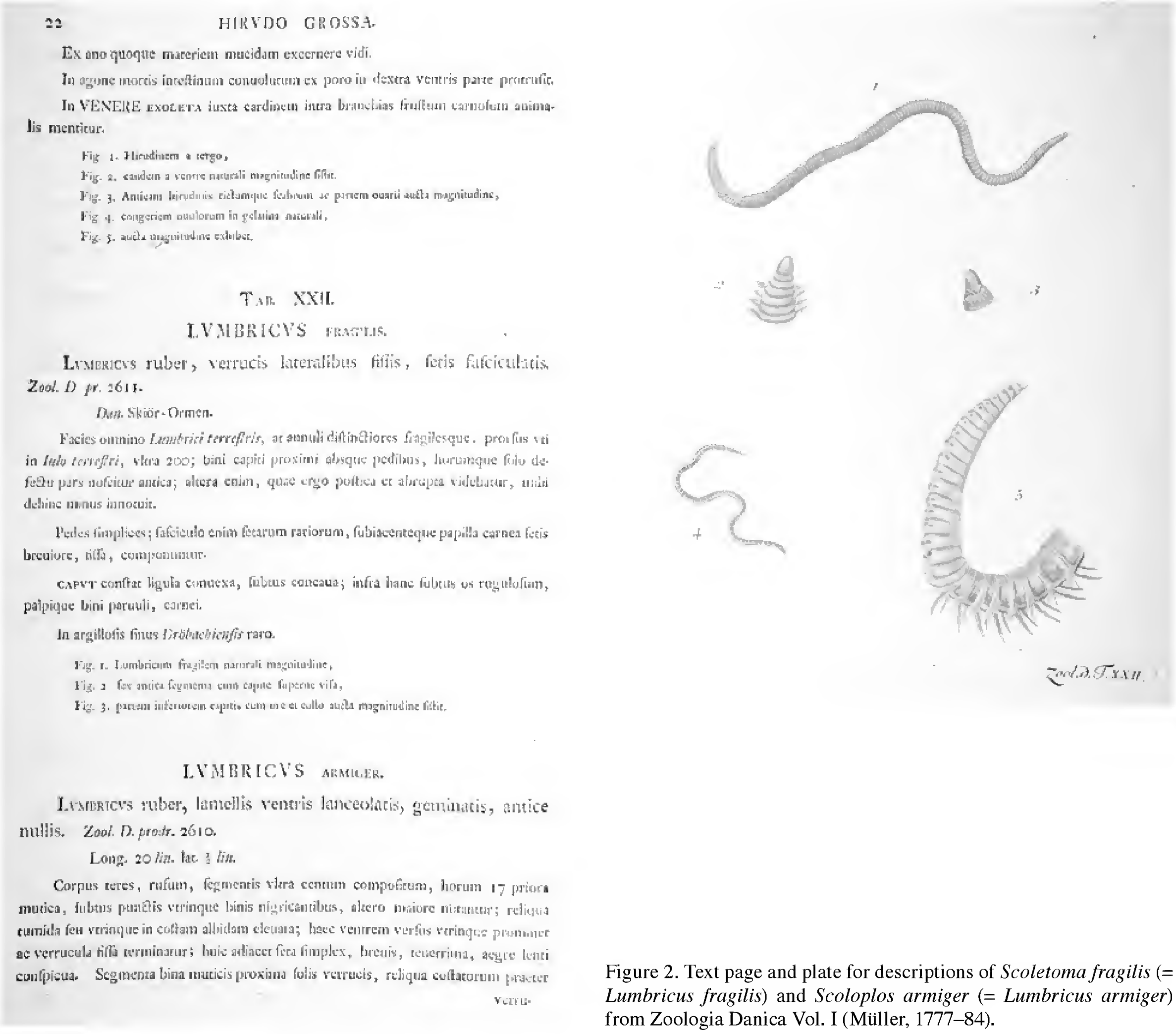
O.F. Müller's most important contribution is the large and ambitious *Zoologia Danica* (complete name *Zoologiae Danica seu Animalium Daniae et Norvegiae rariorum ac minus notorum, Descriptiones et historia* [Descriptions and natural history of the rare and little known animals of Denmark and Norway]), which was intended to include all known animal species in Denmark and Norway. The work was never completed, but four volumes were released (Müller, 1777–84; Müller and Abildgaard, 1789; Müller et al., 1806) before the work was discontinued (Anker, 1950; Wolff, 1994). Müller died soon after the release of the second volume, and the third and fourth volumes were edited and completed by contemporary naturalists in Copenhagen (P.C. Abildgaard, M. Vahl, J. Rathke, H.S. Holten). The text was in Latin, but parallel editions with text in Danish and German were made of the first volume. All species were illustrated by Müller's brother, C.F. Müller, who also edited a new release of the two first volumes in 1788 (Müller, 1788). Fig. 2 presents an example of the quality of the text and illustrations in *Zoologia Danica*.

Prior to the release of *Zoologia Danica*, a so-called forerunner *Zoologia Danica prodromus* was published in 1776 (Müller, 1776). The 'prodromus' is essentially an annotated catalogue of all contemporary known species of animals in Denmark and Norway and the first inventory based on the Linnean classification system. In total, more than 3000 species are included. All species were entered with an access number, scientific name (binomial), brief diagnosis in Latin, references, and vernacular names if appropriate (fig. 3). New species detected by Müller were entered pending a full description in the main work. For several of these, however, no more descriptions were given and the brief and usually very general diagnosis in the 'prodromus' is the only extant information.

For several species described by other authors (e.g. Hans Strøm and Otto Fabricius) and by Müller himself in previous works (Müller, 1771), the scientific name given in the 'prodromus' is the first name published in accordance with the nomenclatural rules and hence the oldest available name of the species. Later, this caused much confusion. One example is the spionid *Spio filicornis* (listed as *Nereis filicornis* in 'prodromus'), which was described by Otto Fabricius from Greenland (Fabricius, 1780). *Spio filicornis* was for a long time considered a European species, but has recently been re-described, based on newly collected material from Greenland (Meissner et al., 2011). This is particularly relevant to determination of type localities for the species, which in several cases are still not settled.

Figure 1. Otto Friderich Müller. From drawing by Cornelius Høyer. Reproduced from Wolff (1994).

A list of valid polychaete species named by O.F. Müller is given in table 1. Müller presented information on sampling localities, mostly as part of the descriptions in *Zoologia Danica*. In some cases details may be found in travel reports and letters. For some species, the sampling locality is exactly specified, but for others, only a general area is indicated. For species cited from other authors, the sampling localities may be found in their descriptions. Tracing type localities may, therefore, be uncertain and requires information from different text sources. For several species, e.g. *Cirratulus cirratus* (Müller, 1776) and *Hediste diversicolor* (Müller, 1776), the type locality has not been clarified. Müller kept a large collection of specimens (Anker, 1950), but no polychaete material is presently known to exist (D. Eibye-Jacobsen, pers. comm.). A more detailed review of the species named by O.F. Müller is in progress and will be published elsewhere.



22 HIRVDO GROSSA.

Ex ano quoque materiem mucidam excernere vidi.

In agone mortis intestinum conuolutum ex poro in dextra ventris parte protrusit.

In VENERE EXOLETA iuxta cardinem intra branchias frustum carnosum animalis mentitur.

Fig. 1. Hirudinem a tergo,
Fig. 2. eandem a ventre naturali magnitudine sistit.
Fig. 3. Anticam hirudinis ciliamque scabrum ac partem ouarii aucta magnitudine,
Fig. 4. congeriem ouulorum in gelatina naturali,
Fig. 5. aucta magnitudine exhibet.

TAB. XXII.

LVMBRICVS FRAGILIS.

LVMBRICVS ruber, verrucis lateralibus fissis, setis fasciculatis. *Zool. D pr.* 2611.

Dan. Skiör-Ormen.

Facies omnino *Lumbrici terrestris*, at annuli distinctiores fragilesque. pro fus vti in *Iulo terrestri*, vltra 200; bini capiti proximi absque pedibus, horumque solo defectu pars noscitur antica; altera enim, quae ergo postica et abrupta videbatur, nulli dehinc manus innocuit.

Pedes simplices; fasciculo enim setarum rariorum, subiacenteque papilla carnea setis breuiore, fissa, componuntur.

CAPVT constat ligula conuexa, subtus concaua; infra hanc subtus os rugulosum, palpique bini paruuli, carnei.

In argillosis sinus *Dröbachiensis* raro.

Fig. 1. Lumbricum fragilem naturali magnitudine,
Fig. 2 sex antica segmenta cum capite superne visa,
Fig. 3. partem inferiorem capitis cum ore et collo aucta magnitudine sistit.

LVMBRICVS ARMIGER.

LVMBRICVS ruber, lamellis ventris lanceolatis, geminatis, antice nullis. *Zool. D. prodr.* 2610.

Long. 20 *lin.* lat. ½ *lin.*

Corpus teres, rufum, segmentis vltra centum compositum, horum 17 priora mutica, subtus punctis vtrinque binis nigricantibus, altero maiore nitantur; reliqua tumida seu vtrinque in costam albidam eleuata; haec ventrem versus vtrinque prominet ac verrucula fissa terminatur; huic adiacet seta simplex, breuis, tenerrima, aegre lenti conspicua. Segmenta bina muticis proxima solis verrucis, reliqua costatorum praeter

verru-

Figure 2. Text page and plate for descriptions of *Scoletoma fragilis* (= *Lumbricus fragilis*) and *Scoloplos armiger* (= *Lumbricus armiger*) from Zoologia Danica Vol. I (Müller, 1777–84).

The second era: Michael Sars and the beginning of systematic descriptions of Norwegian marine fauna

After O.F. Müller, there was a period with few investigations of the Norwegian marine fauna until about 1830, when Michael Sars started his career. From about 1840, several other scientists were active, and the latter half of the century was a very prolific period in the systematic description of the marine fauna (Sakshaug and Mosby, 1996). Michael Sars (1805–1869) was born in Bergen on the west coast of Norway, where he also started his studies of marine animals (fig. 4). He was educated in theology and practiced as a vicar, first in Kinn near Florø (1831–40) and later in Manger near Bergen (1840–54). He was awarded a professorship at the University of Oslo (then Christiania) from 1855, where he remained until his death in 1869 (Økland, 1955; Helle, 2006). Starting in 1849, he made several travels to northern Norway to collect specimens. In Oslo he collected in the Oslofjord with his main focus on the region near Drøbak.

Michael Sars had a broad interest in several marine species groups and early in his career earned an international reputation for studies of the life histories of cnidarians and echinoderms. Throughout his career, he described new species in various groups, among them cnidarians, polychaetes, molluscs and echinoderms. In the 1860s he also sampled, together with his son Georg Ossian Sars, numerous species from the great depths (>800 m) in offshore areas. The deep sea had previously been considered lifeless, and their findings raised a broad international interest in deep-sea expeditions. In Norway the findings contributed to the funding of the Norwegian North Atlantic Expedition, which was carried out in 1876–78 (Sakshaug and Mosby, 1996; Helle, 2006).

Table 2. Chronological overview of polychaete species described by Michael Sars from Norwegian waters. See fig. 6 for localities. NHMO = Natural History Museum Oslo; ZMBN =University Museum of Bergen; ZMBI = Zoological Museum Berlin; NMWC = National Museum of Wales, Cardiff; USNM = National Museum of Natural History, Washington DC.

Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
M. Sars 1829				
Flabelligera affinis	*Flabelligera affinis* M. Sars, 1829	Sars 1829: 31–34, pl. 3, figs 16–19	Bergen area	Original material probably lost
Terebella longicornis		Sars 1829: 28–31, pl. 1, figs 7–9	Bergen area	Uncertain status, original material probably lost
M. Sars 1835				
Terebellides stroemii	*Terebellides stroemii* M. Sars, 1835	1835: 48–50, pl. 13, figs 31a–e	Glesvær near Bergen	Original material lost. Neotype NHMO, selected from Manger near Bergen (Parapar and Hutchings, in press)
Amphitrite gunneri	*Amphicteis gunneri* (M. Sars, 1835)	1835: 50–51, pl. 11, figs 30a–d; 1865: 2–6, 9–10 (offprint)	Glesvær near Bergen; Florø	Lectotype and paralectotype, NHMO (Hartley, 1985). Type locality not specified on label of lectotype (Glesvær and Florø).
Sabella? octocirrata	*Ampharete octocirrata* (M. Sars, 1835)	1835: 51–52, pl. 13, figs 32a–g	Glesvær near Bergen; Florø	Possible syntypes, NHMO (Holthe, 1986)
Serpula libera	*Ditrupa arietina* (O.F. Müller, 1776)	1835: 52–54, pl. 12, figs 33a–c; 1851: 84	Bergen area including Glesvær; Florø	Possible syntypes, NHMO. M. Sars (1835) indicates synonymy with *D. arietina*
Chaetopterus norvegus [sic!]	*Chaetopterus norvegicus* M. Sars, 1835	1835: 54–58, pl. 11, figs 29a–h; 1851: 87; 1861b: 86–87; 1861c: 255–256	Bergen area; Florø	Syntypes, NHMO
Nereis virens	*Alitta virens* (M. Sars, 1835)	1835: 58–60, pl. 10, figs 27a–c	Bergen area	Possible syntypes, NHMO
Phyllodoce foliosa	*Notophyllum foliosum* (M. Sars, 1835)	1835: 60–61, pl. 9, figs 26a–e; 1873a: 224–226	Manger near Bergen	Lectotype and 3 paralectotypes, NHMO (Nygren et al., 2010)
Onuphis conchylega	*Nothria conchylega* (M. Sars, 1835)	1835: 61–63, pl. 10, figs 28a–e; 1851: 89	Bergen area; Florø	Lectotype, NHMO, selected from Florø (Fauchald, 1982)
Polynoë gelatinosa	*Alentia gelatinosa* (M. Sars, 1835)	1835: 63–64, pl. 9, figs 25a–c	Bergen area; Florø	Original material probably lost (Loshamn, 1980)
Nais ? clavicornis	*Macrochaeta clavicornis* (M. Sars, 1835)	1835: 64–65, pl. 9, figs 24a–d	Florø	Original material probably lost (Banse, 1969)
M. Sars 1846				
Oligobranchus roseus	*Scalibregma inflatum* Rathke, 1843	1846: 91–93, pl. 10, figs 20–27; 1863: 52; 1873a	Florø	Holotype, NHMO (Mackie, 1991)
M. Sars 1851				
Notomastus latericeus	*Notomastus latericeus* M. Sars, 1851	1851: 79–80; 1856: 9–12 pl. II, figs 8–17	Florø; Komagfjord	Syntypes, NHMO

Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
Clymene mülleri	*Proclymene muelleri* (M. Sars, 1851)	1851: 80–81; 1856: 13–15, pl. 1, figs 1–7; 1862a: 91 (21–22 in offprint)	Bergen area	Syntypes. NHMO
Clymene cirrosa	?*Euclymene droebachiensis* (M. Sars in G.O. Sars, 1872)	1851: 81	Tromsø	Holotype, NHMO, originally described based on posterior fragment. Possible synonym of *Euclymene droebachiensis* (Arwidsson, 1906)
Ammochares assimilis	*Owenia assimilis* (M. Sars, 1851)	1851: 81–82	Tromsø; Bergen area	Syntypes, NHMO. Species reinstated by Koh et al. (2003)
Sabella crassicornis	*Bispira crassicornis* (M. Sars, 1851)	1851: 82–83; 1862b: 119–121 (28–29 in offprint)	Tromsø	Lectotype, NHMO; paralectotype, ZMBN (Knight-Jones and Perkins, 1998)
Sabella papillosa	*Euchone papillosa* (M. Sars, 1851)	1851: 83; 1862b: 129–130 (38–39 in offprint)	Øksfjord; Havøysund	Syntypes, NHMO
Sabella neglecta	*Potamilla neglecta* (M. Sars, 1851)	1851: 83; 1862b: 122–123 (31–32 in offprint)	Hammerfest; Tromsø	Possible syntypes, NHMO. Neotype (!) selected, ZMBI (Knight-Jones, 1983)
Serpula polita	*Placostegus tridentatus* (J.C. Fabricius, 1779)	1851: 84	Bergen; Øksfjord; Komagfjord	Syntypes, NHMO
Sabellides cristata	*Melinna cristata* (M. Sars, 1851)	1851: 85–86; 1856: 19–24, pl. II, figs 1–7	Bergen; Havøysund	Original material probably lost. Neotype, NMWC, selected from Hjeltefjord near Bergen (Mackie and Pleijel, 1995)
Nerine cirrata	*Laonice cirrata* (M. Sars, 1851)	1851: 87–88; 1862a: 64–65 (15–16 in offprint)	Ure in Lofoten; Tromsø; Hammerfest	Lectotype, NHMO, selected from Ure (Sikorski, 2011)
Nerine foliosa	Possibly synonym of *Scolelepis foliosa* (Audouin and Milne Edwards, 1833)	1851: 87–88; 1862a: 61–64 (12–15 in offprint)	Bergen area	Syntypes, NHMO
Oniscosoma arcticus	*Spinther arcticus* (M. Sars, 1851)	1851: 90; 1862a: 52–55	Komagfjord	Syntypes, NHMO
Euphrosyne armadillo	*Euphrosyne armadillo* M. Sars, 1851	1851: 91; 1862a: 55–56 (6–7, offprint)	Bergen area	Syntypes, NHMO
M. Sars 1856				
Spiochaetopterus typicus	*Spiochaetopterus typicus* M. Sars, 1856	1856: 1–8, pl. I, figs 8–21	Manger (Helle) near Bergen	Syntypes, NHMO
Clymene quadrilobata	*Pseudoclymene quadrilobata* (M. Sars, 1856)	1856: 15–16, pl. II, figs 18–22	Florø; Manger near Bergen	Syntypes, NHMO. Replaced by *Clymene gracilis* new name by Sars (1861c, 1862a). Redescribed as distinct species by Arwidsson (1906)
Sabellides borealis	*Ampharete borealis* (M. Sars, 1856)	1856: 22–24	Reine in Lofoten; Øksfjord	Possible syntypes, NHMO (Holthe, 1986)

Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
Sabellides sexcirrata	*Samytha sexcirrata* (M. Sars, 1856)	1856: 23–24	Manger near Bergen	Possible syntypes, NHMO (Holthe, 1986)
M. Sars 1861a				
Polynoe nodosa	*Eunoe nodosa* (M. Sars, 1861)	1861a: 58–59	Havøysund	Syntypes, NHMO (Barnich and Fiege, 2010)
Polynoe asperrima	*Acanthicolepis asperrima* (M. Sars, 1861)	1861a: 59	Manger and Herdla near Bergen	Syntypes, NHMO C3154 (Barnich et al., 2000)
Polynoe rarispina	*Harmothoe rarispina* (M. Sars, 1861)	1861a: 60	Vadsø	Syntypes, NHMO (Barnich and Fiege, 2009)
Polynoe scabriuscula	*Gattyana cirrhosa* (Pallas, 1766)	1861a: 60–61; 1861c: 252–253; 1869: 254	Kristiansund, Vadsø	Possible syntypes, NHMO. M. Sars (1869) indicates synonymy with *G. cirrhosa*
M. Sars 1861b				
Chaetopterus sarsii	*Chaetopterus sarsii* Boeck in Sars, 1861	1861b: 85–87; 1861c: 255; 1863: 50–51; 1873a: 261–262	Beian in Trondheimsfjord	Syntypes, NHMO. Boeck, 1860: 252 *nomen nudum*
M. Sars 1861c				
Ophiodromus vittatus	*Ophiodromus flexuosus* (delle Chiaje, 1828)	1861c: 255; 1862a: 87–88 (18–19 in offprint); 1873a: 229	Kristiansund, Molde, Manger, Åsgårdstrand in Oslofjord	Type probably lost on loan
Clymene gracilis	*Praxillella gracilis* (M. Sars, 1861)	1861c: 256; 1862a: 91–92 (22–23 in offprint)	Bollærne in Oslofjord; Molde; Kristiansund; Grøtøy and Slåttholmen in Lofoten; Ramfjord near Tromsø; Vadsø	Syntypes, NHMO. *Clymene gracilis* introduced as new name for *Clymene quadrilobata* Sars, 1856. Redescribed as distinct species by Arwidsson (1906)
Clymene biceps	*Chirimia biceps* (M. Sars, 1861)	1861c: 256–258; 1862a: 93–95 (24–25 in offprint)	Bollærne in Oslofjord; Kristiansund; Tromsø; Øksfjord; Vadsø	Syntypes, NHMO
M. Sars 1862a				
Euphrosyne cirrata	*Euphrosyne cirrata* (M. Sars, 1862)	1862a: 56 (7 in offprint); 1863: 50	Manger near Bergen	Possible syntypes, NHMO
Eurythoe borealis	*Pareurythoe borealis* (M. Sars, 1862)	1862a: 58–59 (9–10 in offprint)	Manger near Bergen	Material lost; original description based on notes only (Sars 1862a)
Nerine oxycephala	*Aonides oxycephala* (M. Sars, 1862)	1862a: 64 (15 in offprint)	Florø	Syntypes, NHMO
Castalia aurantiaca	*Hesiospina aurantiaca* (M. Sars, 1862)	1862a: 90 (20 in offprint)	Florø; Manger near Bergen	Lectotype, NHMO, selected from Manger (Pleijel, 2004)
Castalia longicornis	*Hesiospina aurantiaca* (M. Sars, 1862)	1862a: 90 (21 in offprint)	Manger near Bergen	Original material lost. Neotype = lectotype of *H. aurantiaca* (Pleijel, 2004)

Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
M. Sars 1862b				
Dasychone decora	*Branchiomma infarctum* (Krøyer, 1856)	1862b: 124–125 (33–34 in offprint)	Tromsø; Hammerfest; Vadsø	Syntypes, NHMO
Dasychone argus	*Branchiomma bombyx* (Dalyell, 1853)	1862b: 125–126 (34–35 in offprint); 1863: 67–68	Glesvær and Manger near Bergen; Åsgårdstrand in Oslofjord	Syntypes, NHMO
Chone Kröyerii	*Chone kroyerii* M. Sars, 1862	1862b: 126–128 (35–37 in offprint)	Manger near Bergen; Tromsø; Vadsø	Possible syntypes, NHMO. Type material not indicated (Tovar-Hernandez, 2007)
Chone rubrocincta	*Euchone rubrocincta* (M. Sars, 1862)	1862b: 128–129 (37–38 in offprint); 1863: 66–67	Florø; Manger	Syntypes, NHMO (Banse, 1972, Tovar-Hernandez, 2007)
M. Sars 1863				
Polynoë nivea	*Leucia nivea* (M. Sars, 1863)	1863: 39–42	Beian in Trondheimsfjord	Holotype, NHMO (Loshamn, 1980; Chambers, 1989; Barnich and Fiege, 2010)
Polynoë clavigera	*Harmothoe clavigera* (M. Sars, 1863)	1863: 42–46	Kristiansund	Holotype, NHMO (Barnich and Fiege, 2009)
Polycirrus trilobatus	*Amaeana trilobata* (M. Sars, 1863)	1863: 53–58	Slåttholmen in Lofoten, Kristiansund	Syntypes, NHMO
Terebella artifex	*Lanice conchilega* (Pallas, 1766)	1863: 58–66	Beian in Trondheimsfjord	Syntypes, NHMO
M. Sars 1865a				
Amphicteis finmarchica	*Ampharete finmarchica* (M. Sars, 1865)	1865a: 10–14 (6–10 in offprint)	Ramfjord near Tromsø	Syntypes, NHMO
Polycirrus arcticus	*Polycirrus arcticus* M. Sars, 1865	1865a: 14–16 (10–13 in offprint)	Tromsø; Vadsø	Possible syntypes, NHMO (Holthe, 1986)
Terebella ebranchiata	*Leaena ebranchiata* (M. Sars, 1865)	1865a: 16–20 (13–16 in offprint)	Varangerfjord	Possible syntypes, NHMO (Holthe, 1986)
M. Sars 1867 (*nomina nuda*)				
Clymene laeviceps				
Lophosyllis maculata				
M. Sars 1869 (*nomina nuda*)				
Maldane? pellucida				
Eumenia? erucaeformis				
Trophonia pallida	Possibly synoym of *Diplocirrus glaucus* (Malmgren, 1867)			Synonymy indicated by M. Sars (1869)
Trophonia pilosa				
Pygophelia singularis				

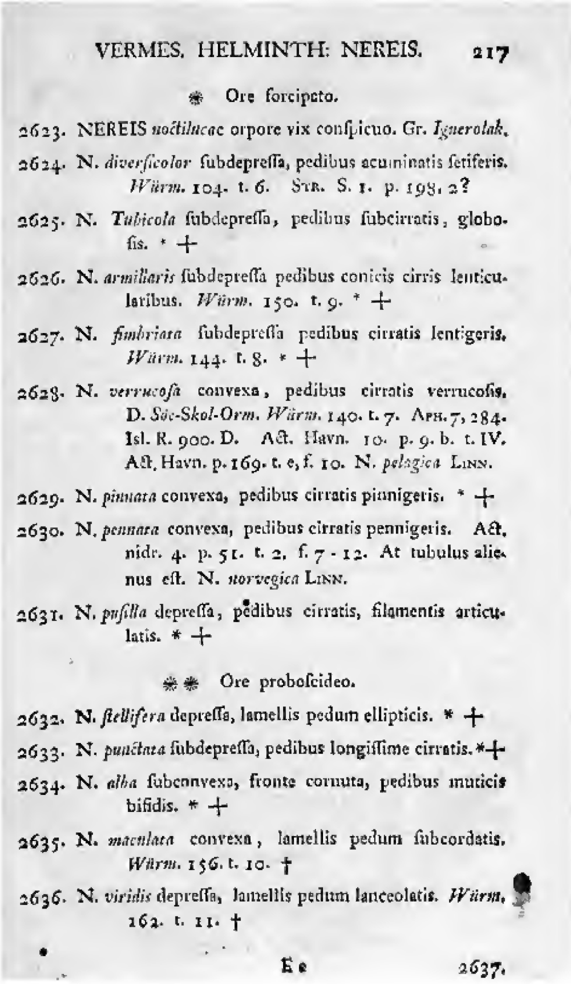
Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
Polynoe abyssicola	*Harmothoe abyssicola* Bidenkap, 1894		Skråva in Lofoten, Oslofjord	Syntypes, NHMO. Described by Bidenkap (1894). Revised Barnich and Fiege (2009) on specimens from Oslofjord
M. Sars in G.O. Sars 1872a				
Paramphinome pulchella	*Paramphinome jeffreysii* (McIntosh, 1868)	1872a: 45–49, pl. 4, figs 19–35.	Lofoten, Oslofjord, Ålesund near Molde	Possible syntypes, NHMO. M. Sars, 1869: *nomen nudum*
Umbellisyllis fasciata	Possibly synonym of *Odontosyllis gibba* Claparède, 1863 (Nygren 2004)	1872a: 41–43, pl. 4, figs 12–18	Flekkefjord near Kristiansand, Lofoten, Hardangerfjord, Kristiansund	Type material not confirmed. M. Sars 1869: *nomen nudum*
M. Sars in G.O. Sars 1872b				
Laenilla mollis	*Austrolaenilla mollis* (M. Sars in G.O. Sars, 1872)	1872b: 406–407; 1873a: 207–214, pl. 14, figs 1–16	Drøbak in Oslofjord	Type probably lost. Extended description (1873a) includes specimens from Lofoten
Eteone fucata	Possibly synonym of *Eteone flava* (Fabricius, 1780) (Pleijel 1993)	1872b: 407; 1873a: 226–229, pl. 15, figs 1–6	Drøbak in Oslofjord	Syntypes, NHMO. M. Sars 1867: *nomen nudum*
Onuphis quadricuspis	*Paradiopatra quadricuspis* (M. Sars in G.O. Sars, 1872)	1872b: 407–408; 1873a: 216–222, pl. 15, figs 7–19	Drøbak and Åsgårdstrand in Oslofjord; Skrova in Lofoten	Lectotype, NHMO, selected from Drøbak (Fauchald, 1982). M. Sars, 1867: 291; 1869: *nomen nudum*
Aricia norvegica	*Phylo norvegica* (M. Sars in G.O. Sars, 1872)	1872b: 408; 1873a: 236–240, pl. 16, figs 1–8	Bolærne and Drøbak in Oslofjord; Lofoten	Syntypes, NHMO. M. Sars 1867: 291 *nomen nudum*
Trophonia flabellata	*Pherusa flabellata* (M. Sars in G.O. Sars, 1872)	1872b: 409; 1873a: 249–252, pl. 17, figs 1–12	Drøbak in Oslofjord; Skrova and Brettesnes in Lofoten	Syntypes, NHMO. M. Sars 1869: *nomen nudum*
Chloraema pellucidum	*Flabelligera affinis* M. Sars, 1829 (fide Støp-Bowitz 1948)	1872b: 409–410; 1873a: 252–261, pl. 16, figs 9–20	Not specified, whole coast	Holotype, NHMO (Støp-Bowitz, 1948). M. Sars 1867: 291: *nomen nudum*, as *Siphonostomum pellucidum;* 1869: *nomen nudum*, as *Chloraema pellucidum*
Prionospio plumosus	*Prionospio plumosa* (M. Sars in G.O. Sars, 1872)	1872b: 410; 1873a: 263–268, pl. 17, figs 13–29	Drøbak in Oslofjord	Types, USNM (Sigvaldadottir, 1998). M. Sars 1867: 291 *nomen nudum*, as *Ctenospio plumosus*
Spiophanes cirrata	Possibly synonym of *Spiophanes kroyeri* Grube, 1860 (Söderström 1920; Meissner 2005)	1872b: 410–411; 1873a: 268–273, pl. 18, figs 1–16	Drøbak in Oslofjord; Skrova in Lofoten	Type probably lost (Meissner, 2005)
Clymene planiceps	*Isocirrus planiceps* (M. Sars in G.O. Sars, 1872)	1872b: 411–412	Drøbak in Oslofjord, Terøy in Hardanger	Syntypes, NHMO

Original name	Valid name	References, including later descriptions	Original localities	Type material and remarks
Clymene Dröbachiensis	*Euclymene droebachiensis* (M. Sars in G.O. Sars, 1872)	1872b: 412	Drøbak in Oslofjord	Syntypes, NHMO
Clymene affinis	*Praxillella affinis* (M. Sars in G.O. Sars, 1872)	1872b: 412	Bolærne in Oslofjord	Syntypes, NHMO
Lumbriclymene cylindricauda	*Lumbriclymene cylindricauda* M. Sars in G.O. Sars, 1872	1872b: 413	Drøbak in Oslofjord	Syntypes, NHMO. M. Sars 1867: 291 *nomen nudum*, as *Clymene cylindricauda*
Streblosoma cochleatum	*Streblosoma bairdi* (Malmgren, 1866)	1872b: 414	Drøbak in Oslofjord	Possible syntypes, NHMO
Streblosoma intestinale	*Streblosoma intestinale* M. Sars in G.O. Sars, 1872	1872b: 414	Drøbak in Oslofjord; Odvær in Lofoten	Possible syntypes, NHMO
Thelepodopsis flava	*Thelepus cincinnatus* (Fabricius, 1780)	1872b: 415	Drøbak in Oslofjord	Possible syntypes, NHMO
Chone longocirrata	*Chone longocirrata* M. Sars in G.O. Sars, 1872	1872b: 415–416	Drøbak in Oslofjord	Type probably lost (Tovar-Hernandez, 2007)
Dasychone inconspicua	*Branchiomma inconspicuum* (M. Sars in G.O. Sars, 1872)	1872b: 416	Drøbak in Oslofjord	Syntypes, NHMO. M. Sars 1867: 291 *nomen nudum*
Protula borealis	uncertain, possibly synonym of *Protula tubularia* (Montagu, 1803)	1872b: 417	Not specified, whole coast	Syntypes NHMO. M. Sars 1865b: *nomen nudum*; 1866: *nomen nudum*; 1869: *nomen nudum*

Table 3. Summary of polychaetes described from Norwegian waters in the 19th century by several authors: Heinrich Rathke, Anders Ørsted, Georg Ossian Sars, Lauritz Esmark, Gerhard Armauer Hansen and Wilhelm Storm. See tables 1 and 2 for species described by O.F. Müller and Michael Sars. NNHE, Norwegian North-Atlantic Expedition 1876–78; NHMO, Natural History Museum Oslo; NTNU-VM, Norwegian University of Science and Technology, University Museum Trondheim; ZMBN, University Museum of Bergen. See fig. 6 for localities.

Original name	Localities	Remarks
Rathke 1843		
Sigalion idunae	Molde	Synonymised with *Sthenelais boa* (Johnston, 1833)
Nereis grandifolia	Kristiansund	Synonymised with *Nereis pelagica* Linnaeus, 1758
Nereis sarsii	?	Synonymised with *Hediste diversicolor* (O.F. Müller, 1776)
Syllis cornuta	Kristiansund	Accepted
Syllis tigrina	Molde	Synonymised with *Syllis armillaris* (O.F. Müller, 1776)
Halimede venusta	Molde	Synonymised with *Nereimyra punctata* (O.F. Müller, 1776)
Ephesia gracilis	Molde	Synonymised with *Sphaerodorum flavum* (Ørsted, 1843)
Aricia muelleri	Molde	Synonymised with *Scoloplos armiger* (O.F. Müller, 1776)
Arenicola boeckii	Trondheimsfjord	Synonymised with *Arenicolides ecaudata* (Johnston, 1835)
Scalibregma inflatum	Molde	Accepted; neotype from Molde (Mackie, 1991)
Ammotrypane aulogaster	Drøbak in Oslofjord; Molde and Namsenfjord	Synonymised with *Ophelina acuminata* Ørsted, 1843
Ammotrypane limacina	Molde	Accepted as *Ophelia limacina*
Ammotrypane oestroides	Molde	Synonymised with *Travisia forbesii* Johnston, 1840
Siphonostoma vaginiferum	Kristiansund	Accepted as *Flabelligera vaginifera*
Siphonostoma villosum	Molde	Accepted as *Brada villosa*
Siphonostoma inhabile	Molde	Accepted as *Brada inhabilis*
Clymeneis stigmosa	Kristiansund and Molde	Accepted
Ørsted 1845		
Sigalion tetragonum	Drøbak in Oslofjord	Accepted as *Neoleanira tetragona*
Syllis longocirrata	Drøbak in Oslofjord	Accepted as *Syllides longocirrata*
Notophyllum polynoide	Drøbak in Oslofjord	*Nomen dubium*, original material lost (Nygren et al., 2010)
Goniada norvegica	Drøbak in Oslofjord	Accepted
Spione trioculata	Drøbak in Oslofjord	?
G.O. Sars 1873b		
Nychia globifera	Storegga, off Western Norway	Accepted as *Harmothoe globifera*. Type lost (Barnich and Fiege, 2010)
Hermadion? hyalinus	Storegga, off Western Norway	Accepted as *Adyte hyalina*; holotype, NHMO (Bock et al., 2010)
Esmark 1874		
Eteonopsis geryonicola	Oslofjord	Accepted as *Ophryotrocha geryonicola*, syntypes NHMO
Hansen 1879a		
Polynoë aspera	NNHE stn 48	Accepted as *Harmothoe aspera*; type ZMBN
Polynoë (Eunoë) islandica	NNHE stn 48	Synonymised with *Eunoe nodosa* (M. Sars, 1861); type ZMBN
Nephthys atlantica	NNHE stns 18, 31 and 87	Synonymised with *Aglaophamus malmgreni* (Théel, 1879); type ZMBN

Original name	Localities	Remarks
Typhlonereis gracilis	NNHE stn 40	Accepted: lectotype, ZMBN 2183 (Bakken, 2003)
Onuphis hyperboräa	NNHE stn 18 and 48	Accepted as *Nothria hyperborea*; lectotype, ZMBN 2210, NNHE stn 18 (Fauchald, 1982)
Scalibregma (?) abyssorum	NNHE stn 18	Nomen dubium (Bakken et al., 2014), type ZMBN
Scalibregma parvum	NNHE stns 18 and 31	Accepted as *Pseudoscalibregma parvum;* lectotype ZMBN, NNHE stn 31 (Bakken et al., 2014)
Ammotryphane cylindricaudatus	NNHE stns 31 and 87	Accepted as *Ophelina cylindricaudata;* lectotype ZMBN, NNHE stn 87 (Kongsrud et al., 2011)
Späerodorum abyssorum	NNHE stn 33	Accepted as *Ephesiella abyssorum*: type ZMBN
Trophonia hirsuta	NNHE stns 18 and 31	Accepted as *Diplocirrus hirsutus*; type ZMBN
Cirratulus abyssorum	NNHE stn 87	Uncertain status; type ZMBN
Cirratulus abranchiatus	NNHE stn 31	Accepted as *Chaetozone abranchiatus*
Clymene Koreni	NNHE stn 87	Accepted as *Maldane koreni*; type ZMBN
Myriochele Sarsii	NNHE stn 38, 40 and 51	Synonymised with *Myriochele heeri* Malmgren, 1867; type ZMBN
Potamilla Malmgreni	NNHE stn 40 and 51	Accepted as *Potamethus malmgreni*; type ZMBN
Protula arctica	NNHE stn 51	Accepted as *Protis arctica*; type ZMBN
Hansen 1879b		
Polynoë arctica	NNHE stn 223, 224, 237	Synonymised with *Eunoe oerstedi* Malmgren, 1866; type ZMBN
Aricia arctica	NNHE stn 224, Jan Mayen	Accepted as *Scoloplos arctica*; type ZMBN
Storm 1879		
Lænilla violácea	Røberg in Trondhjemsfjord	Accepted as *Leucia violacea*; syntypes NTNU-VM (Barnich and Fiege, 2009)
Lænilla oculinarum	Galgenes in Trondhjemsfjord	Accepted as *Harmothoe oculinarum*. Type specimens in NHMO and NTNU-VM (Fiege and Barnich, 2009).
Hansen 1880		
Polynoe assimilis	NNHE stn 363	Synonymised with *Harmothoe globifera* (G.O. Sars, 1873), (Barnich and Fiege, 2010); type ZMBN
Polynoe spinulosa	NNHE stn 363	Synonymised with *Eunoe nodosa* (M. Sars, 1861); type ZMBN
Polynoe foraminifera	NNHE stn 338	Synonymised with *Eunoe nodosa* (M. Sars, 1861); type ZMBN
Polynoe glaberrima	NNHE stn 366	Accepted
Trophonia borealis	NNHE stns 270, 275	Synonymised with *Pherusa plumosa* (O.F. Müller, 1776); type ZMBN
Trophonia rugosa	Spitzbergen, Magdalenabay	Accepted as *Brada rugosa*; type ZMBN
Trophonia arctica	Spitzbergen, Magdalenabay	Synonymised with *Brada rugosa* (Hansen, 1880)
Brada granulosa	NNHE stn 337	Accepted; type ZMBN
Myriochele danielsseni	NNHE stn 192	Accepted; type ZMBN
Storm 1881		
Leodice gunneri	Trondhjemsfjord	Synonymised with *Eunice norvegica* (Linnaeus, 1767)



VERMES. HELMINTH: NEREIS. 217

✻ Ore forcipato.

2623. NEREIS *noctilucae* orpore vix conſpicuo. Gr. *Ignerolak*.

2624. N. *diverſicolor* ſubdepreſſa, pedibus acuminatis ſetiferis. *Würm.* 104. t. 6. Str. S. 1. p. 198. 2?

2625. N. *Tubicola* ſubdepreſſa, pedibus ſubcirratis, globoſis. * +

2626. N. *armillaris* ſubdepreſſa pedibus conicis cirris lenticularibus. *Würm.* 150. t. 9. * +

2627. N. *fimbriata* ſubdepreſſa pedibus cirratis lentigeris. *Würm.* 144. t. 8. * +

2628. N. *verrucoſa* convexa, pedibus cirratis verrucoſis. D. *Söe-Skol-Orm. Würm.* 140. t. 7. Aph. 7, 284. Isl. R. 900. D. Act. Havn. 10. p. 9. b. t. IV. Act. Havn. p. 169. t. e, f. 10. N. *pelagica* Linn.

2629. N. *pinnata* convexa, pedibus cirratis pinnigeris. * +

2630. N. *pennata* convexa, pedibus cirratis pennigeris. Act. nidr. 4. p. 51. t. 2. f. 7-12. At tubulus alienus eſt. N. *norvegica* Linn.

2631. N. *puſilla* depreſſa, pedibus cirratis, filamentis articulatis. * +

✻✻ Ore proboſcideo.

2632. N. *ſtellifera* depreſſa, lamellis pedum ellipticis. * +

2633. N. *punctata* ſubdepreſſa, pedibus longiſſime cirratis. * +

2634. N. *alba* ſubconvexa, fronte cornuta, pedibus muticis bifidis. * +

2635. N. *maculata* convexa, lamellis pedum ſubcordatis. *Würm.* 156. t. 10. †

2636. N. *viridis* depreſſa, lamellis pedum lanceolatis. *Würm.* 162. t. 11. †

E e 2637.

Figure 3. Example of text page from *Zoologia Danica prodromus* for polychaetes with armed mouth ('ore forcipato') and with eversible pharynx ('ore proboscideo'). From Müller (1776).

Figure 4. Michael Sars. Photography by P.M. Thomsen. Reproduced from Økland (1955).

Michael Sars described nearly 80 species of polychaetes, of which 54 are considered valid (table 2). The descriptions generally had a standardised form, with a diagnosis in Latin followed by an extended description with morphological details in Norwegian. In some few cases, descriptions were given in either German (Sars, 1846) or French (Sars, 1856). Some of the works were re-edited and translated into German, French or English and published in international journals (see Sars, 1829, 1835, 1856, 1869). From about 1860, most new species were published as contributions from the newly established scientific society of Christiania (Det norske Videnskaps-Akademi [The Norwegian Academy of Science and Letters]). His latest descriptions of new species were published after his death in three papers edited (without changes) or revised by his son Georg Ossian Sars (Sars, 1872a, 1872b, 1873a). Altogether, there are 14 publications with descriptions of new species of polychaetes (table 2).

The correct reference to the descriptions needs attention. Several contributions from the scientific society were published both in an annual periodical and as separate offprints. The offprints had separate pagination (starting at p. 1) and usually a different title (e.g. Sars, 1862a, 1862b). The periodical was published the year after the presentations, e.g. contributions for 1861 were published in 1862. It may also cause problems that several species were described more than once. This was the case for some species for which the first publication was rather short and Michael Sars then presented a more complete description in a later publication. The use of illustrations varied. The earliest publications were illustrated (Sars, 1829, 1835, 1846, 1856), but later publications were generally not. The last species descriptions (Sars, 1873a) contained detailed illustrations of some of the species made by G.O. Sars, who was an extremely skilled illustrator.

Contemporary with Michael Sars, several foreign naturalists visited Norway for fauna studies. In approximately 1840, new species were described by the Danish naturalist Anders Ørsted and the German–Polish naturalist Heinrich Rathke (table 3). Ørsted visited Drøbak, having been inspired by the works of O.F. Müller (Ørsted, 1845), whereas Rathke visited several places in the middle part of Norway (Rathke, 1843). No material from Ørsted's polychaetes from Drøbak is known to exist (Wolff and Petersen, 1991). The existence of the material of Rathke is uncertain. A couple of decades later,

the most important contribution to the knowledge of the polychaete fauna was recorded by Gerhard Armauer Hansen in his treatment of the material collected during the Norwegian North-Atlantic Expedition (NNHE), 1876–78. In total 27 polychaete species were described as new species from the expedition, of which 16 are considered valid (table 3). All species descriptions were originally published in Norwegian (Hansen, 1879a; 1879b; 1880), but the descriptions were later repeated with parallel text in English in a comprehensive expedition report (Hansen, 1882).

Museum collections of original material

In general the material collected by the early naturalists were kept in their own private collections or donated or sold to museum collections (Anker, 1950; Økland, 1955). In the museums, collected specimens were placed in common collections. Specimens and samples used for species descriptions were generally not specifically indicated. The degree to which original specimens have been identified and catalogued as ‘types’ at some later stage varies among museums. All too often, however, it seems that original materials have been forgotten and/or overlooked in the collections and consequently been reported as missing when asked for in modern taxonomic studies. For most early-described species, the identification of original material (holotype or syntypes) today is, therefore, totally dependent on information on sample labels (site, date, collector) and knowledge of the original sampling sites. The present principles of designating and cataloguing a type series as specified in the Zoological Code (ICZN) did not come into force until much later (ICZN, 1999).

In Norway, there are four natural history museums that maintain scientific marine collections. The first to be established was the collections of the Royal Norwegian Society of Sciences and Letters in Trondheim, which was founded in 1760 (Moen, 2006; Bakken et al., 2011). The other museums, in Oslo (then Christiania), Bergen and Tromsø, were founded in 1812, 1825 and 1872, respectively. In their first periods of activity, the museums concentrated on local fauna and flora, but gradually the museums also built up collections of specimens from other parts of Norway, and, starting in the 1870s, from expeditions to the Nordic Seas and Arctic areas and more distant destinations (see e.g. Sakshaug and Mosby, 1996). Some specimens have been distributed among the museums as early curators seemed to share or split samples between the museums (Bakken, 1999).

In the present study, efforts have been made to identify original materials from Michael Sars in Norwegian museums that have not yet been identified as ‘types’. Most of the material is located in the collections of the Natural History Museum, University of Oslo (NHMO), but some is also found in the University Museum, University of Bergen (ZMBN). During his research, Michael Sars also sent specimens to other European museums, e.g. in Copenhagen (information from letters, see Økland, 1955). Potentially, original material (syntypes) may, therefore, have been distributed among several museums. In the present study, original material from 25 species has been identified in the collections of the museum in Oslo (see table 2). Original labels with Michael Sars’ characteristic hand-writing (fig. 5) and corresponding information on sampling sites from labels and species descriptions have been taken as evidence for the status of the material. These specimens have now been catalogued and transferred to a separate type collection. Material of somewhat uncertain status, e.g. lacking original labels, has been registered as possible types (table 2) and catalogued.

Type localities

The Zoological Code (ICZN) states that all sampling localities for a collection of syntypes are to be regarded as type localities (ICZN, 1999). When a lectotype has been designated, or a neotype in the case of missing original material, the locality of the designated specimen is the sole type locality, and localities for other previous syntypes lose their status. These specifications imply that a uniquely defined type locality (one locality only) will be the case only for species originally described from one locality or when a lectotype or neotype has been designated in later revisions. For modern taxonomy, and for molecular studies of species complexes in particular, the precise location of one type locality is crucial. With regard to the species described by O.F. Müller, some species included in *Zoologia Danica* were described from one locality, which then fixes the type locality (e.g. Drøbak in the Oslofjord for *Scoletoma fragilis*, *Eunice pennata* and *Hyalinoecia tubicola*: table 1). For Müller’s other species, especially those that referred to other authors in the ‘prodromus’, the identification of sampling localities may be more obscure. As Müller in the ‘prodromus’ often referred to several authors and publications, the first step is to decide which of them constitutes the original description; then information may be extracted on localities, which are often rather inaccurately reported. The matter is also complex for poorly characterised species that essentially have been diagnosed by later authors, e.g. *Glycera alba* by Ørsted (1843), based on specimens from sampling localities outside of the area indicated by Müller.

The naturalists of the 19th century generally reported their sampling localities, but often rather roughly, with little more than place name and depth. The studies of Ørsted (1845) and Rathke (1843) were restricted to one or a few places. Michael

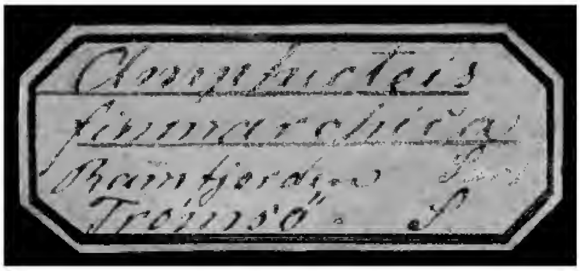


Figure 5. Original label written by Michael Sars for *Ampharete finmarchica*. Original text reads: ‘Amphicteis finmarchica Sars. Ramfjorden Tromsö S.’ Natural History Museum, Oslo.

Sars, however, often reported several localities for his species, especially in the late publications, when he had collected material from all parts of Norway (table 2, fig. 6). In the descriptions, he did not indicate whether material from one or several localities had been used. Therefore, it should be a task in connection with revisions to critically examine all syntypes and select lectotypes that are in accordance with the species descriptions. Until today this has only been done for seven of the species of Michael Sars (table 2). Presently, there is one specified type locality for only about half of the species that he described as new, either by original designation (one locality) or by subsequent selection of a lectotype or neotype by later authors.

Conclusions

The correct taxonomy of the species is the key to biological knowledge and the very basis for documenting biodiversity. Taxonomy requires a thorough knowledge of past research, even if that means beginning with old, poorly preserved and labelled specimens. It is acknowledged that modern research is hindered by the inaccessibility of older taxonomic literature, poor descriptions of early-described species, and the uncertain existence and location of type material (Glasby and Read, 1998). The present rapidly increasing use of molecular genetic methods for species characterisation reinforces the need to clearly assess the identity of the species. Any information on original material, their repositories and sampling localities is therefore urgently needed. In Norway, correct taxonomy is critical for biodiversity mapping (e.g. the MAREANO seabed mapping program: Buhl-Mortensen et al., 2012), environmental surveillance monitoring at offshore petroleum installations, and studies of the effects of climate changes. Furthermore, recent studies of selected polychaete families have revealed considerable species shifts from offshore shelf to deep-water areas in the Nordic Seas (Kongsrud et al., 2011; Bakken et al., 2014).

The present study is intended to facilitate access to descriptions, material and localities of the early-described species from Norway. Most of the old literature is in Danish or Norwegian, with place names that often are obsolete or very local. Native knowledge is therefore essential, as is knowledge of the history of science, reading descriptions in the original language, tracing unpublished field notes and letters that may be kept as part of collections, and access to museum catalogues to supplement more precise data on sampling localities. Knowledge of local geography is also of paramount importance, especially when place names have changed over time with the development of language and change of local administrative systems.

Basic taxonomy incorporating revisions of early-described species is tedious work. It is a real challenge to do revisions fast enough to keep up with molecular studies. In cases where molecular data are needed at the first instance, the best practice will be to collect specimens from original localities or within the geographical range where the original material may have been collected, which implies that information on original sampling and material must be known. The documentation of material and sampling localities of the early-described species is thus a basis for the advancement of taxonomy and biodiversity mapping using new techniques and methods.

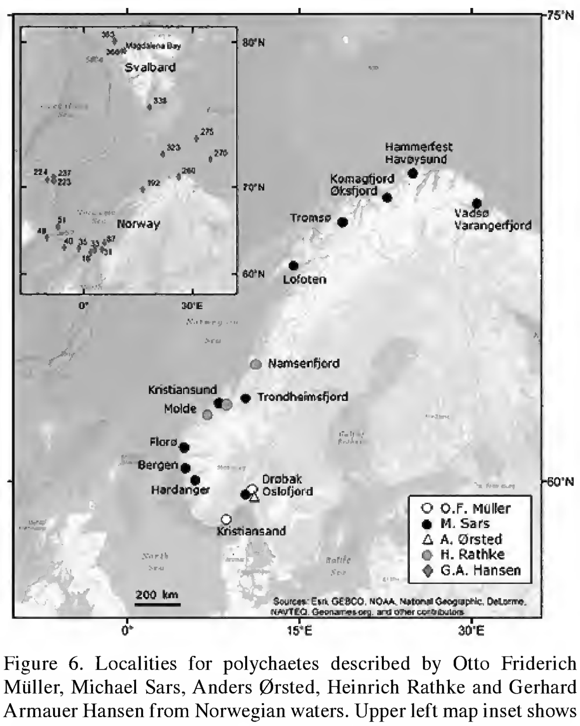


Figure 6. Localities for polychaetes described by Otto Friderich Müller, Michael Sars, Anders Ørsted, Heinrich Rathke and Gerhard Armauer Hansen from Norwegian waters. Upper left map inset shows stations sampled by the Norwegian North-Atlantic Expedition (NNHE) 1876–1878.

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The pros and cons of using micro-computed tomography in gross and micro-anatomical assessments of polychaetous annelids

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Abstract

Paterson, G.L.J., Sykes, D., Faulwetter, S., Merks, R., Ahmed, F., Hawkins, L.E., Dinley, J., Ball, A.D. and Arvanitidis, C. 2014. The pros and cons of using micro-computed tomography in gross and micro-anatomical assessments of polychaetous annelids. *Memoirs of Museum Victoria* 71: 237–246.

The use of micro-CT scanners in the study of anatomy and functional morphology of marine invertebrates is becoming more common. The advantages and disadvantages of this methodology for the study of the internal anatomy of polychaetes are discussed. Soft-bodied invertebrates such as polychaetes pose some specific problems. It can be difficult to gain sufficient contrast between different types of tissues to be able to image them with X-rays. A range of stains can help enhance the contrast between tissues. In this study we investigate a number of stains, concentrating on those considered reversible. The advantages of such stains in the study of museum specimens and the resulting possibilities for large-scale comparative morphology studies are outlined.

Keywords Polychaeta, internal anatomy, micro-CT, staining methods

Introduction

Phylogenetic studies of polychaetous annelids in recent years have mainly used molecular approaches (e.g. Struck et al., 2011; Wiklund et al., 2008; Zrzavý, et al., 2009). Less numerous but of equal importance have been those studies that have used recent methodological advances in morphological techniques, such as confocal microscopy to study nerves and muscles systems (e.g. Mao, 2007; Orrahage, 1990; Zanol et al., 2011; see reviews in Lanzavecchia et al., 1988; Purschke, 1988; 2005; Saulnier-Michel, 1992; Tzetlin and Purschke, 2005; Tzetlin and Zhadan, 2009). It may be argued that further progress in understanding the phylogeny of polychaetes and other taxa requires the pace of morphological work to quicken to match the rapidity of molecular investigations. Anatomical studies are more intensive in terms of the time needed, skills required and techniques involved. Undertaking large-scale anatomical studies can be a daunting task, not least of which is access to the necessary comparative

material. And yet "…it is the history of morphological change that we wish to explain…" (Raff et al., 1989, quoted in Nielsen, 2012).

The development and increasing availability of micro-computed tomography (micro-CT) scanners holds great promise in supporting structural and functional anatomical analyses (e.g. Golding et al., 2007; Li et al., 2008). CT scans have been used in medical fields for many years and their ability to produce 3-D renderings of many features is well known and documented (e.g. Udupa and Herman, 2000). Their use in anatomical studies of non-human subjects is increasing and there have been several studies focused on polychaetes. For example, Dinley et al., (2009) showed how the method could be used in functional anatomical studies, while Faulwetter et al. (2013) have shown how the rendered micro-CT images provide detailed taxonomic results.

There is no doubt that this is a maturing technology but what is perhaps the most exciting aspect of using micro-CT is that images of internal structures can be obtained without damage to the specimen. The technology provides the opportunity to undertake large-scale studies in a relatively short timescale and using museum collections not normally amenable to conventional anatomical studies. Nevertheless, because the technology is still emerging, questions need to be asked as to the efficacy of the approach, what it can and, as importantly, what it cannot as yet visualise, and from a curator's perspective that the method is safe to use on specimens.

In this paper we will: 1) evaluate micro-CT as a method for the study of internal anatomy of polychaetes; 2) assess the pros and cons of the various approaches that are possible using this methodology; and, 3) by way of example, present some preliminary results based on a study of the internal anatomy of the pharyngeal apparatus of 'errant' polychaetes (sensu Struck et al., 2011) re-examining the seminal work of Dales (1962). This study complements that of Faulwetter et al. (2013), focussing on the use of micro-CT for internal anatomical studies.

Material and methods

CT technology

Two different micro-CT scanners have been used to scan the polychaetes in this study. 1) Nikon metrology HMX ST 225 at the Imaging and Analysis Centre, Natural History Museum (NHM). The HMX ST 225 uses either a tungsten, molybdenum, silver or copper target and has a 4 megapixel (2000x2000 pixel) detector panel. The highest possible resolution is 5μm/pixel. The scanner can produce X-ray energies of up to 225kV and 200μA. 3,142 projections are taken over a 360° rotation and subsequently reconstructed with CT Pro software (Nikon Metrology, Tring, UK), which uses a modified Feldkamp's back-projection algorithm.

2) SkyScan 1172 microtomograph at the Hellenic Centre for Marine Research uses a tungsten source and is equipped with an 11 megapixel CCD camera (4000×2672 pixel). The highest possible resolution is 0.8 μm/pixel. Specimens were scanned at a voltage of 60 kV with a flux of 167μA without filter and scans were performed for a full rotation of 360°. Images were acquired at highest camera resolution. The projection images were subsequently reconstructed into a sequence of cross sections with the NRecon software (Bruker/SkyScan, Kontich, Belgium) which uses a modified Feldkamp's back-projection algorithm. These cross-sections were reconstructed from the full set of projection images (360°), other reconstruction parameters were chosen individually for each sample.

Three-dimensional models were created, from the tomographic datasets, and manipulated using the Drishti software suite (http://code.google.com/p/drishti-2) Limaye and VG-Studio Max 2.1 (Volume Graphics GmbH, Heidelberg, Germany). Drishti is recommended for the manipulation of this type of dataset. Drishti operates by loading a stack of 'back-projected' images (cross-sections of the sample) from the scan then converting it into 3-D volumetric data. This image is composed of voxels (3-D pixels) that are individually assigned a grayscale value, which represents the x-ray absorption at that point.

Staining protocols

Stains such as phosphotungstic acid (PTA) and iodine are well established in micro-CT studies (see Metscher, 2009) and appear to have similar general properties. As part of a wider study on the use of micro-CT in the study of polychaete anatomy we reviewed the potential for existing histological stains to be developed for use in CT studies. Specifically, we were looking for stains known to highlight particular tissues and which also have the potential to increase the absorption of X-rays by those tissues, making them appear more opaque. The test determined how easy the protocols for staining were, the specificity of the stain in CT rendering and whether the process could be reversible, making them more amenable to use on museum specimens. In addition to Iodine and PTA, two traditional histological stains which stain specific tissues, were tested – silver stain (Golgi, 1873) and iron stain (Wigglesworth, 1952). The former stains nerve tissue while the latter highlights nucleic acids and proteins. As part of a Master's study project undertaken by one of the authors (RM) the efficacy of the various stains were assessed for a number of different staining and clearing regimes. Standard histological methods were used to assess stain penetration and using the results the timings and concentrations cited below were derived.

a) Silver stain. The Silver stain method is based on the method of Golgi (1873) but adapted as a bulk stain. Stain reversal is possible.

1) Specimens were stained in 3% aqueous potassium dichromate for up to seven days, and the solution replaced daily and kept in the dark. 2) Excess solution was removed and samples placed in a solution of 2% silver nitrate and stained for seven days; the solution was changed frequently until brown precipitate no longer appeared. The specimen will be red to black in colour. 3) Specimens were removed from the stain, rinsed with, and then stored in, 70% ethanol, ready to be scanned.

Stain removal. 1) Specimens were dehydrated, firstly in 90% ethanol for 24 hours then a further 24 hours in 100% ethanol. 2) Specimens were placed in a 1:1 solution of hexamethyldisilizane (HMDS) and 100% ethanol for 24 hours, then placed in 100% solution of HDMS for 24 hours. 3) Once the stain had disappeared the specimen was rehydrated in stages back to 70% ethanol.

Table 1. Comparison of traditional anatomical approaches, using the SEM and micro-CT.

	CT Approach	Classical
Serial section	Straightforward	Process well understood but histology can be complex
Dissection	Virtual–straightforward after training	Needs skill and manual dexterity
3-D Reconstruction	Easy–depending on equipment	Involved
Identification of anatomical feature/tissue	Difficult at times	Well established
Impact on specimen	Specimen available for further study	Specimen altered and in some cases destroyed
DNA impact	Limited depending on X-ray dosage	Compromised in some procedures
Resolution	Micron/submicron range	Thin sections can give high cell-level resolution but resolution in Z is generally compromised

HMDS should always be used in a fume cupboard and be handled with protective gloves and goggles.

b) Iron Stain. Wigglesworth (1952) developed the Iron stain to highlight and measure the abundance of nucleic acids and proteins. Exact timing will depend on specimen size. The method outlined below applies to large specimens (>3 cm in length), in this case a large nereidid.

1) Specimens were hydrated in stages to distilled water. 2) Placed in 0.25% solution of ammonium iron (III) sulphate (iron alum) for five minutes. 3) Rinsed gently with distilled water. 4) Placed in 10% solution of ammonium sulphide for 150 seconds (*this was carried out in a fume cupboard*). A black iron sulphide precipitate formed immediately. 5) Specimen were then blotted dry and transferred to 2% solution of potassium ferricyanide. A cloudy precipitate formed. The solution was changed until this no longer happened. 6) Specimens were left in final solution for 24 to 48 hours (changing the solution after 24 hours if longer staining was sought until specimens were a blue colour. 7) Finally the specimens were rinsed with and stored in 70% ethanol, ready to scan.

Stain removal. 1) Specimen placed in saturated solution of potassium oxalate for at least 48 hours until all the blue stain has been removed. The solution should be replaced every 24 hours. Stain removal can take up to one week on large specimens. 2) Specimen can then be dehydrated in stages back to 70% ethanol.

c) Iodine stain. Exact timing will depend on the size of specimen. These instructions are for a large nereidid. 1) Specimens were dehydrated to 100% ethanol, in two steps 80% then 100%, 24 hours per step. 2) They were then placed in I2E (stock solution of 1% metallic iodine in 96% alcohol) for 24 hours, ready for scanning.

Stain removal. 1) Specimens were placed in 90% ethanol for as long as it took to remove stain. As the stain comes out of the specimen The solution was replaced, at least every 24 hours as it became cloudy black, until the precipitate no longer formed2) Specimen was hydrated back to 70% ethanol in stages.

d) Phosphotungstic acid (PTA). The stock solution comprised 1% (w/v) phosphotungstic acid in water. The specimens were stained in a mixture of 30 ml 1% PTA solution and 70 ml absolute ethanol (0.3% solution).

1) Specimens were dehydrate to 70% ethanol (PTA in 70% ethanol keeps indefinitely). 2) They were stained for at least 2 hours but longer (e.g. overnight) was sometimes necessary depending on specimen size. 3) Specimens were then washed in 70% ethanol. Staining is stable for months. 4) Specimens were scanned in 70% – 100% ethanol.

This is an irreversible stain.

Enhancing contrast by use of (HMDS).

Hexamethyldisilizane (HDMS) removes water from tissues effectively increasing the clarity of boundaries between air and tissue which in turn enhances the contrast when scanning with X-rays. The use of HMDS emulates critical point drying and has therefore gained favour in scanning biological material using the SEM (Bray et al., 1993). However, the standard method (Oshel, 1997) has had to be adapted for polychaete specimens to be scanned using a micro-CT.

1) Specimens were dehydrated through ethanol series 70%, 80%, 90% to 100% with 24 hours in each. 2) Then transferred to 1:1 solution of 100% ethanol and HMDS for 24 hours. 3) Transferred to HMDS for at least 24 hours. 4) Specimen was removed from solution and air dried overnight in a fume cupboard. Specimen is then ready for scanning.

Rehydrating. 1) The procedure reversed the above starting with the 100% HMDS with at least 24 hours in each solution until the desired storage solution was reached.

Results

General approach

Table 1 contrasts conventional anatomical methods employing such techniques as dissection and traditional histology with micro-CT. Both approaches have drawbacks. A conventional approach involves a range of techniques to produce data. Dinley (2013) demonstrated the range of approaches useful in functional anatomical studies of polychaetes. Such techniques range from the low resolution – gross anatomy provided by dissections – to ultra high-resolution derived from transmission electron microscopy. Reconstructing the structure of particular features or visualising the arrangements of internal anatomy is time consuming and can be compromised by artefacts caused by sample processing, for examples wrinkles and shrinkage in the sections can distort anatomical features and the resolution in thin sections might be very good in X and Y, but the sections are considerably thicker than this in conventional serial sections, so the data collected is not isotropic whereas computed voxels from micro-CT are isotropic. In addition to the range of skills required in developing this conventional anatomical 'pipeline', such studies also require the use of a number of specimens. Therefore, large-scale comparative studies are particularly challenging to undertake. Access to museum specimens, an obvious source of specimens from a broad range of species, is restricted because of damage resulting from destructive sampling, dissections and alteration of specimens in making serial sections.

By contrast, micro-CT scans and supporting software allow the researcher to perform many tasks virtually, such as dissection or sectioning in various planes as well as produce accurate 3-D rendering of anatomical features without any induced distortion. Using the micro-CT scanner overcomes many of the issues which restrict the use of specimens from museum collections and opens the way for relatively rapid yet detailed anatomical studies.

However, micro-CT also has challenges and problems. In this next section we will outline some of these issues and discuss the solutions or alternatives.

Issues and problems

X-ray transparency and anatomical imaging. Problems associated with trying to image soft-bodied invertebrates, such as polychaetes, stem from the fact that they absorb almost no X-rays, resulting in images with very little contrast. Whilst jaws and other hard structures such as chaetae can be visualised, other internal features such as nerves, muscles and blood vessels can be more challenging to discriminate.

With a low contrast image, internal anatomy may be difficult to describe or illustrate accurately. Unstained material poses particular problems as the images can be 'noisy' and lengthy manipulation with visualization software is needed to differentiate real structures from rendering artefacts (fig. 1). So it may be necessary to assess structures and features observed in micro-CT images by comparing them to classical anatomical studies in the initial stages.

There are approaches which can overcome, at least to some extent, the problem of X-ray transparency. Dependent on the scanner used, parameters (e.g. scanning time, filters, X-ray energy, wavelength) can be optimised to reduce noise and increases the contrast and so improve the final images.

Studies using the NHM micro-CT scanner on unstained polychaetes suggest that using a molybdenum target with exposure times of 354 ms and voltages of 110 kV at 200 μA and exposure times of 354 ms produces good quality images. Good images were obtained using the Skyscan 1172 with a tungsten target, 60kV / 167μm without filter (or with an aluminium filter if the specimen contains both hard and soft structures). In both cases the specimens were scanned in a sealed tube in air, and not immersed in liquid medium (a small reservoir of liquid at the bottom of the tube kept the specimens hydrated).

Resolution. Classical histological analyses making use of embedded and serially sectioned materials deliver high spatial resolution compared to many micro-CT scanners. It is possible to scan to relatively high resolutions using the micro-CT but this is dependent on the on the type of CT scanner employed. With the classical "cone beam" micro-CT scanner the spot size determines the maximum resolution possible and the geometry of the scanning system (origin of the X-rays; position of the sample; position and size of the detector panel; number and size of pixels in the panel) determine the maximum size of specimen that can be examined for any given spot size or resolution. An approximate guide is that the higher the resolution required, the smaller the area of the sample that can be scanned. Alternative micro-CT systems make use of X-ray focussing systems, lenses and detector panels derived from Synchrotron X-ray micro-CT technologies and these systems can overcome many of the limitations of the cone-beam scanners, but at increased cost and complexity.

Use of stains in anatomical studies. While stains described here increase the opacity of tissues, most are non-specific, unlike conventional histological stains which have a long history of study and many can be tissue or cell-type specific. Most stains currently employed in micro-CT analyses are used to enhance the bulk contrast rather than distinguishing between specific tissues. Thus it is often the case that distinctly different tissues appear to have the same or similar contrast in the resulting images. Also, bulk staining poses problems in that the stain has to be able to penetrate the specimen and still be of sufficient molecular weight to absorb effectively X-rays. Specimens stored in alcohol or dehydrated in various mediums have poorer permeability than fresh material. There are methods to 'relax' fixed tissue which increases the permeability of the cuticle but these have yet to be tested on polychaete specimens. Conventional histochemical stains are used on very thin sections of tissue so that penetration is not usually an issue.

Staining for specific tissues

Silver stain. Results indicate that the main drawback with Silver stain is that is does not penetrate effectively far within the tissues when used as a bulk stain. The stain is difficult to use and unstable in that it often does not stain but precipitates out of solution. Generally, whilst in some cases it has been shown to stain nerves, overall the resulting images are 'noisy' showing poor resolution (fig.1a). These results contrast with those of Butzloff (2011) for honey bees, where silver was used to good effect to stain a number of internal features. It is likely that the better results obtained are due to the chemistry of

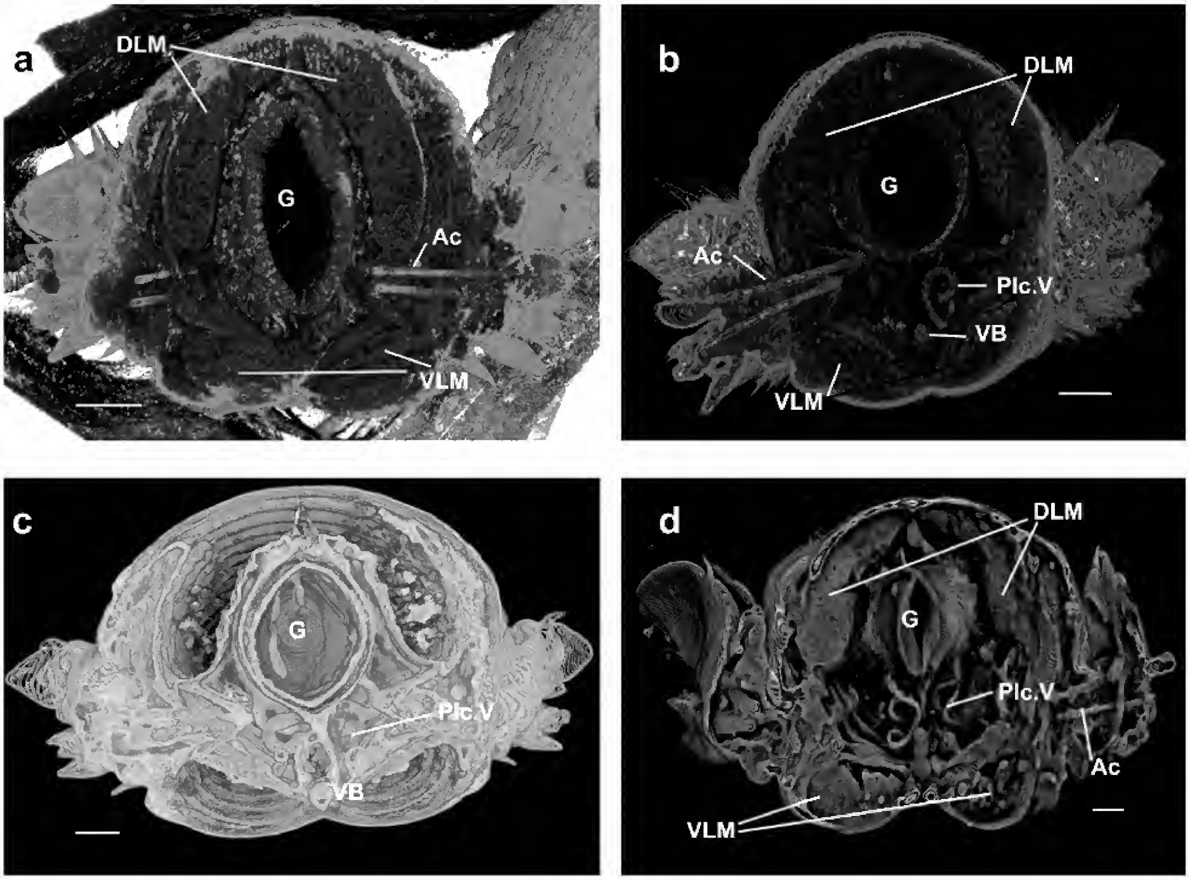

Figure 1. Transverse sections of *Hediste diversicolor* after treatment with reversible stains or drying agents. a) Silver stain, the gut and main muscle blocks can be seen but also showing paper material used to stabilise the specimen surrounding the central image (molybdenum target, 131 KV, 354 millisec exposure; b) iron stain, again gut and main muscles can be seen but also ventral blood vessels linking the central ventral blood vessel to the network surrounding the gut (molybdenum target, 131 KV, 500 millisec exposure); c) Iodine shows similar anatomical features as Iron stained material (molybdenum target, 130 KV, 320 millisec exposure); d) Hexamethyldisilizane (HDMS) image shows more clearly the internal anatomy including the ventral blood vessels (molybdenum target, 110 KV, 300 millisec exposure). Scale bar = 1.00 mm. Specimens were scanned using the Nikon metrology HMX ST 225 at the NHM. Abbreviations: Ac–internal paradpodial acicula; DLM–dorsal longitudinal muscle; G–gut; Plc-V–Plexus lateral connective blood vessels; VB–ventral blood vessel; VLM–ventral longitudinal muscles

chitin and silver but also due to action taken to improve the permeability and therefore uptake of silver. Chemically enhancing permeability through the epidermis is potentially also a useful area for future investigation for polychaetes.

Iron stain. Results of Iron staining showed more promise than the Silver stain. Surface features were clear and internal features generally showed greater contrast (fig.1b). Blood vessels were clearly identified in nereidids and arenicolids. This method is also reversible by placing the specimen in a saturated solution of potassium oxalate until the original blue stain disappears.

Iodine stain. Metscher (2009) described a range of methods using iodine to stain soft tissue. The ease of use and levels of contrast obtained have made this a popular method in micro-CT scanning. With polychaetes results are less consistent. For example, this stain works well with those species with well-developed muscle systems such as nereidids (fig. 1c) but is less successful with groups such as arenicolids where muscle systems are less concentrated. The method is also easily reversible by placing the specimen in 90% ethanol until the iodine is removed from the specimen.

PTA stain. Phosphotungstic acid worked very well on all studied specimens (fig.2). Muscles and the cuticle stained very well, a known feature of PTA, which binds preferentially to certain proteins (Quintarelli et al., 1973). However, PTA penetrates tissues slowly and is bound in high quantities, so staining can take several weeks for large specimens and the solution needs to be renewed frequently until the desired staining effect is achieved.

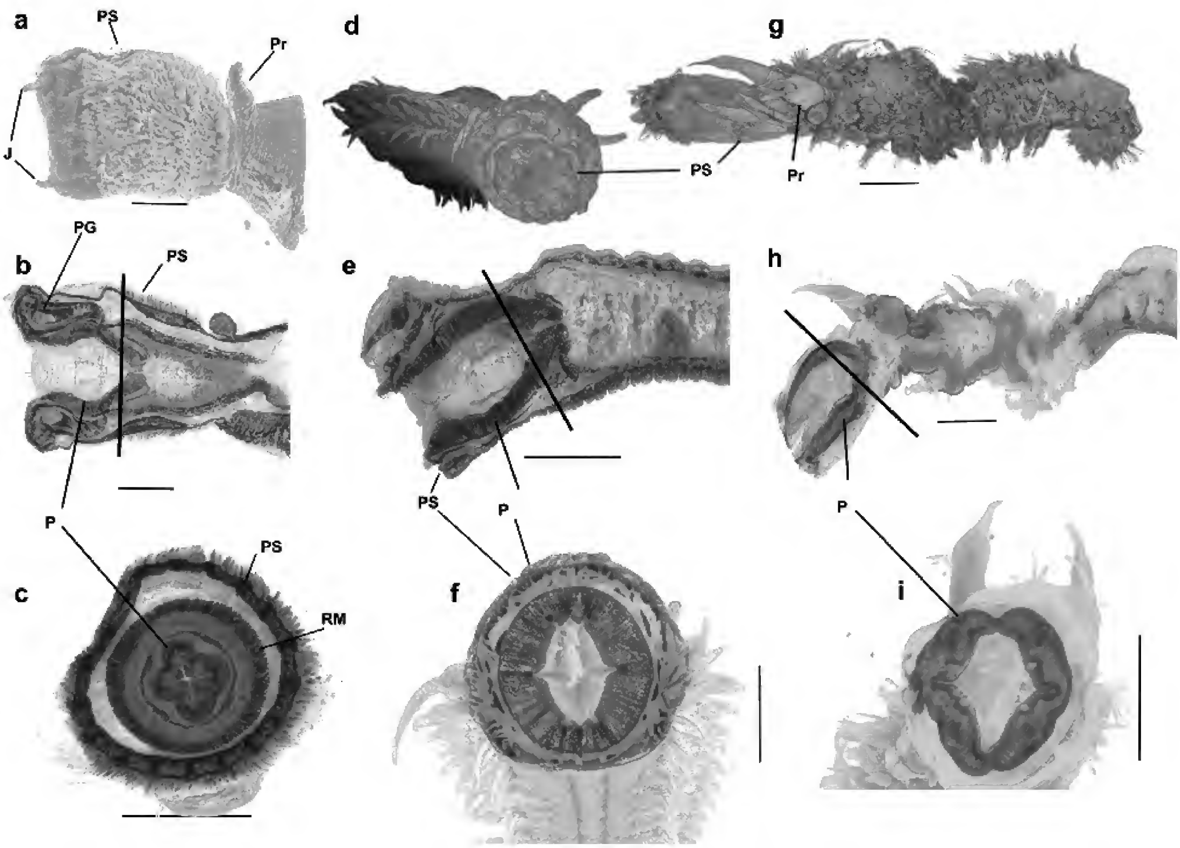

Figure 2. Pharyngeal anatomy of Glyceridae: *Glycera tesselata* (PTA-staining) (a–c); Pilargidae: *Sigambra parva* (d–f) and Polynoidae: *Lepidonotus clava* (g-i). *Glycera* a) surface morphology showing everted pharynx; b) longitudinal section through everted pharynx; c) transverse section of gut as indicated by line in b); scale bars = 0.5 mm. *Sigambra* d) surface morphology showing everted morphology; e) longitudinal section through the pharynx; f) transverse section through distal pharynx as indicated by the line in e); scale bars = 0.5 mm. *Lepidonotus* g) surface morphology showing everted pharynx; h) longitudinal section through pharynx; i) transverse section through distal pharynx as indicated by line in h); scale bar = 1.00 mm. P = pharynx. All three examples show a relatively short axial pharynx approximately as wide as long. The distal part of the pharynx is characterised by distinct muscle blocks which when contracted form a cruciform cross section. Specimens were scanned using the SkyScan 1172 microtomograph at HCMR at 60kV / 167μA, without a filter, no camera binning, full rotation of 360°, tungsten target. Abbreviations used: J–jaws; P–pharynx; PG–poison glands; Pr–prostomium; PS–proboscidian sheath; RM–ring muscle.

Creating greater contrast by drying

Protocols using Hexamethyldisilizane (HMDS) are gaining increased use in electron microscopy and micro-CT because of the greater clarity and contrast of the resulting data. HMDS effectively mimics the critical-point drying process, dehydrating the tissues and, as importantly, this drying process appears to be reversible with limited after effects on the specimen. Fig. 1d shows how effective HDMS can be. Fine scale internal anatomy such as the lateral connective blood vessels are clearly seen as are the dorsal and ventral blood vessels themselves. Muscular tissue is well differentiated and a reasonable degree of resolution is possible. However, internal tissue damage is possible, particularly tearing and ruptures, caused by differential drying during the dehydration process in HDMS. Specimens treated with HMDS become fragile and can be damaged if not handled carefully.

Further work needs to be undertaken to ensure that tissue damage either is not a problem or that a suitable protocol can be established to minimise these effects.

An example of the uses of micro-CT in the study of polychaete anatomy

Dales' (1962) seminal paper laid out the fundamental gross anatomy of the polychaete pharynx and, whilst there have been a number of revisions of parts of this schema, a comprehensive review of this work has yet to take place. Using both micro-CT scanners, we have scanned the pharyngeal anatomy of a representative species from most families currently considered

to be part of the Aciculata clade (*sensu* Rouse and Pleijel, 2001). The basic gross morphology was assessed. A list of the species examined is given in the figure captions. Figs 2-4 indicate that there are significant differences in the overall proportions of the pharynx and associated structures. Fig. 2 illustrates what might be termed taxa with a short pharynx, i.e one where the length to breadth ration is 1:1 or 2:1. The relative proportions of the pharynx varies from being relatively short and approximately as wide as long in the glycerid, pilargid and polynoid. Fig. 3 shows taxa where the pharynx is much longer than broad i.e. >3:1. The hesionid and phyllodocid have long pharynxes while the nephtyid has an intermediate length. The pharynx among taxa shown in fig. 4 have different anatomical arrangements. In the syllid the basic pattern of a thin mucular tube (sensu Dales 1962) connecting to a thick muscular pharynx was not observed. The muscles of the buccal tube in the syllid are not well developed, and this region could be better described as a proboscidain tube leading to a muscular proventicle (sensu Tzetlin and Purschke, 2005).

Dales (1962) proposed that the muscular pharynx in *errant* taxa was used primarily to crush prey. A second character found in some families is the development of four sets of longitudinal muscle blocks in the distal part of the pharynx (Figs 2, 3) resulting in a cruciform cross-section. Dinley et al. (2009) showed this arrangement in Nephtyidae (*Nephtys hombergi,* fig. 3i), suggesting that it facilitated the crushing of ingested prey. Other families showing this arrangement are the Glyceridae (fig. 2c), Pilargidae (fig. 2f), and the scaleworm families Polynoidae (fig.2i), Sigalionidae (not shown here) and Aphroditidae (not shown here). It was absent in the Hesionidae (fig3c), Phyllodocidae (fig.3e), Syllidae and Nereididae specimens examined (fig. 4).

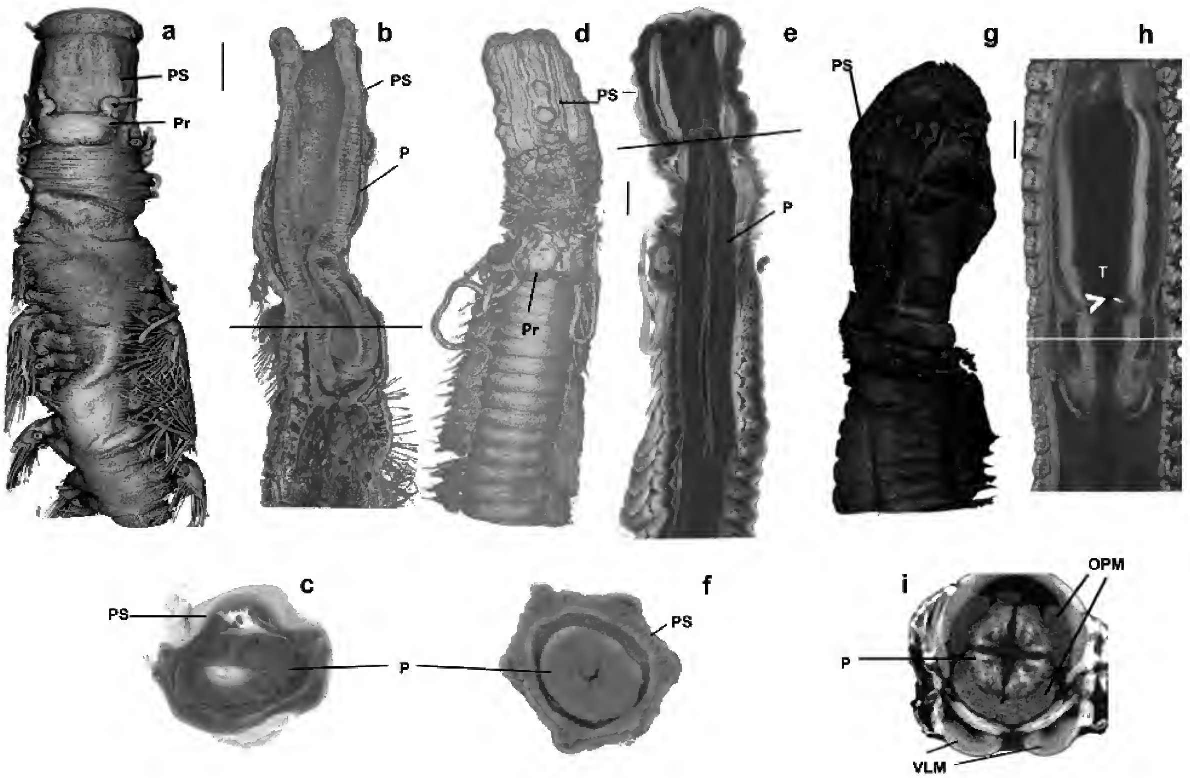

Figure 3 Pharyngeal anatomy of Hesionidae: *Hesiospina similis* (a-c, PTA staining); Phyllodocidae: *Phyllodoce lineata* (d-f, PTA staining) and Nephtyidae *Nephtys hombergi* (g, Iron stain, h-i, unstained). *Hesiospina* a) surface morphology showing everted pharynx; b) section through everted pharynx; c) TS showing distal pharynx as indicated by line in b. *Phyllodoce* d) surface morphology showing everted pharynx; e) section through pharynx; f) TS showing distal pharynx as indicated by line in e. Scale bars = 0.5 mm. *Nephtys* images from three different individuals g) surface morphology showing everted pharynx; h) section showing pharynx but not everted; i) TS of distal pharynx indicated by line in h. Scale bar = 1.00 mm *Hesiospina* and *Phyllodoce* have long thin pharynges while *Nepthys* has a medium lengthed phaynx. Only *Nepthys* shows the cruciform muscle arrangement in the distal pharynx, in the others the muscles do not appear to form these discrete blocks. Images 3a–f were produced using the SkyScan 1172 microtomograph at HCMR at 60kV / 167μA, without a filter, no camera binning, full rotation of 360°, tungsten target. Images 3g–i were produced using the Nikon metrology HMX ST 225 at the NHM (60 KV, 2 sec exposure, molybdenum target.) Abbreviations used: OPM–outer pharyngeal muscles; P–pharynx; Pr–prostomium; PS–proboscidian sheath; T–teeth; VLM–ventral longitudinal muscle.

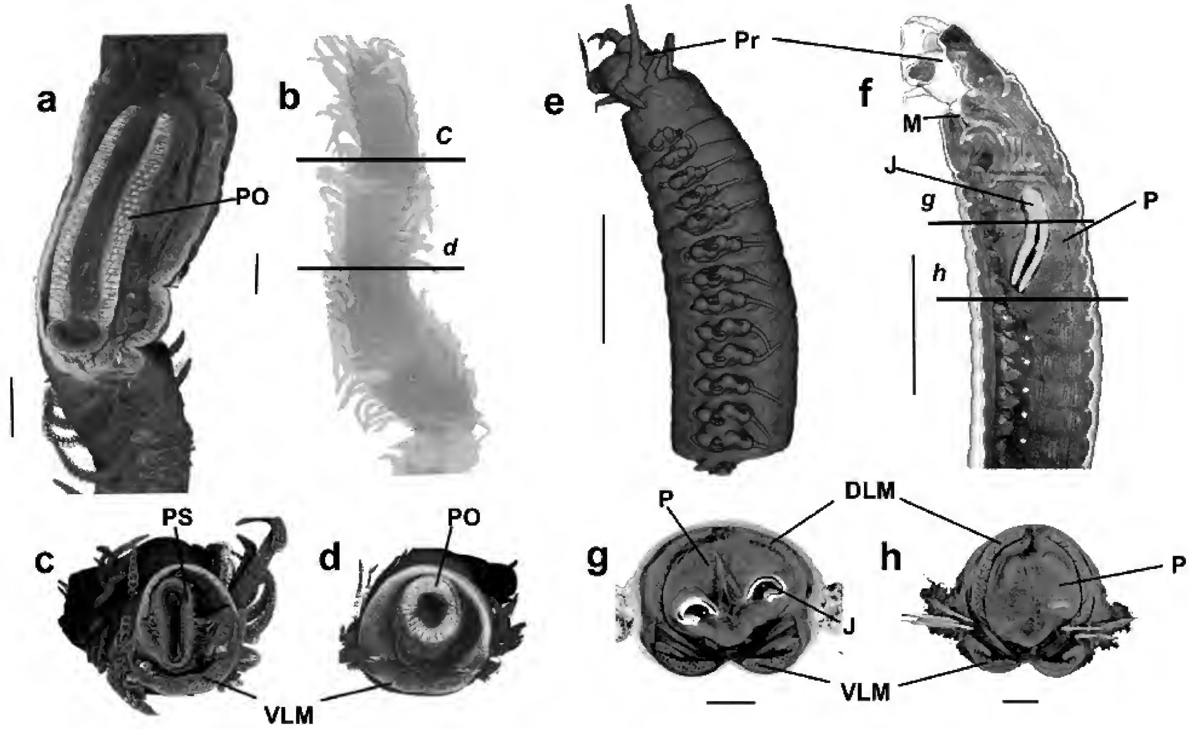

Figure 4. Pharyngeal anatomy of Syllidae: *Syllis gracilis* (a-c, PTA stained) and *Hediste diversicolor* (d-h,). *Syllis* a) section through body showing the proventriculus; b) surface morphology, lines *c* where transverse section c image taken, line *d* where transverse section d image taken; c) TS showing pharyngeal tube; d) TS showing proventricle. Scale bars = 0.5 mm. *Hediste* e) surface morphology; f) section through pharynx, lines g and *h* where transverse section images taken; g) TS through anterior pharynx at level of jaws; h) TS through distal pharynx. TS through pharynx indicates that the pharynx is not symmetrical, particularly in the distal part. Scale bars e, f = 5.00 mm, g,h = 1.00 mm. Images 1a–d were produced using the SkyScan 1172 microtomograph at HCMR at 60kV / 167μA, without a filter, no camera binning, full rotation of 360°, tungsten target. Images i-h were produced using the Nikon metrology HMX ST 225 at the NHM (60 KV, 2 sec exposure, molybdenum target). Abbreviations used:; DLM–dorsal longitudinal muscles; J–jaws; M–mouth; P–pharynx; Pr–prostomium; PO–proventricle; PS–proboscidian sheath; VLM–ventral longitudinal muscles.

Finally, while most of the families examined showed a symmetrical or nearly symmetrical axial pharynx, Nereididae did not. (fig. 4h). There was a distinct asymmetry with the ventral muscle blocks more developed than the dorsal (also noted by Dales, 1962). This arrangement may be related to the orientation of the large jaws, a feature absent in most other families.

Analyses of the gross anatomy is subject of continuing study but it appears that the stomodeum and associated structures can produce more characters for phylogenetic studies than have been used the past.

Discussion

Micro-CT is an imaging tool *par excellence*. Table 2 summarises the advantages and disadvantages of using micro-CT in anatomical studies. The advantages centre around the ease of studying specimens without damaging them and the relative ease of interpreting resulting images. A range of techniques can be deployed to produce virtual dissections of key features and serial sections in any plane desired. The resulting files, both original image stacks and rendered images are standard image files and thus can be distributed without compatibility problems between researchers. CT rendering can also be embedded within PDFs (see Faulwetter et al. 2013 for an example), which enables readers to examine and interact with the images produced. It is also possible that rendered images of type specimens could be sent as virtual loans instead of delicate specimens.

Despite the apparent high capital costs (conventional cone-beam scanners range from US$80K to over US$400K depending on the features), scanners are actually comparable in price with highly specified traditional compound microscopes in the case of the cheaper scanners; while the more expensive scanners are comparable with scanning electron microscopes, thus bringing CT scanning within reach of many institutions. Questions regarding the resolution of the resultant images depend on the specimens and to some degree the techniques employed, particularly whether staining is used. However, technological advances in instrument design are resulting in greater resolution (Stock, 2012).

Table 2. Comparing the advantages and disadvantages of imaging with a micro-CT.

Pros
MicroCT is relatively quick to scan – 40 minutes to 12 hours (overnight)
Specimens are available for future study
Ease of reconstruction and investigation
Volumes created can be distributed and reanalysed easily
Images are easy to interpret and display – 2-D and 3-D
Using a range of techniques it is possible to use Types and rare specimens
Micro-CT scannere are becoming relatively inexpensive (less than the cost of a SEM)
Free analytical software exists (e.g. Drishti, Image J)
Cons
Lack of stains for specific tissues
Image volumes are large (>3+ GB)
Rendering the images is very time consuming depending on what you want to achieve
Storage and retrieval of large numbers of files
Data pipelines and IT infrastructure can be an issue
Technical support helps enormously in running and developing techniques

Attempts to develop stains to highlight specific tissues have mixed success and more development is needed – a topic which is of interest to other disciplines as well (Pauwels et al., 2013). Drying with HMDS appears to provide a useful procedure to enhance tissue contrast in soft-bodied invertebrates like polychaetes. However, there is some development still needed on the methodology to understand the risk posed by differential drying which can result in tissue damage.

Perhaps the most important considerations when embarking on micro-CT studies are the time and infrastructure required. The amount of time is dependent on two distinct aspects of the study. The first is the degree of detail and discrimination required, while the second depends on the IT infrastructure and support available. The first is driven by the scientific question and is mediated by factors such as the need for contrast enhancement, the resolution of the micro-CT scanner, the x-ray source, etc., as explained above. High resolution studies will require more effort in adjusting the initial parameters than those undertaken to look at gross anatomical features and can only be undertaken on small samples. Micro-CT studies can be considered as analysis-heavy. It is relatively quick to acquire the X-ray images needed to create the reconstruction but it then requires a reasonable investment of time to process the images into a coherent and recognisable result. While powerful software is available, some free, it nevertheless takes time to produce images of specific tissues or structures. Rendering of surface features and anatomy is easiest to undertake but generating pictures of internal anatomy can involve considerable manipulation of the rendered images to isolate and display specific features. The results are, however, considerably easier for third parties to interpret in resulting publications and the data files are available allow others to manipulate, explore and evaluate the data produced.

Consideration must also be given to data management when undertaking micro-CT studies. In laboratories with existing imaging capability such data pipelines will be well established but for individuals and newly established micro-CT systems, consideration must be given to the transfer, retrieval, manipulation and long-term storage of files. An image stack of X-rays is often gigabytes in size (depending on specimen size and how much of the specimen is imaged). Manipulating and analysing such files requires a powerful computer with considerable RAM (read-only memory) size and dedicated graphics card. Individual scientists need to consider how they will store original images and rendered results and will need access to a secure off-site server for long-term storage.

One aspect of the use of X-rays is their potentially damaging effect on genetic tissue. Given that X-rays are a core tool for human medicine, this suggests that use of micro-CT may have limited impact on genetic material. Trials using bird specimens did not find any discernible effects (Paredes et al., 2012) and tests on polychaete material also failed to show any major impact, at least for the 16S rRNA gene (Faulwetter et al., 2013). These results indicate that – at least with commonly used scanning parameters – there should be no impediment to using this approach on specimens in museums.

Conclusions

The current state-of-the-art suggests that the micro-CT is a particularly useful tool for anatomical studies, particularly for large-scale comparative projects. In conjunction with other methods, the micro-CT data are also useful in isolating specific areas or internal structures for further studies.

New instruments, software and processors mean that the technology is advancing and that increased use will advance

our understanding of the anatomy at increasingly higher resolutions. Real time functional anatomical analyses will also be possible. Thus, the potential for polychaete anatomical studies has never been so great, and three-dimensional imaging techniques such as micro-CT have the potential to give a strong boost to the discipline and pave the way for new discoveries.

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Systematics, evolution and phylogeny of Annelida – a morphological perspective

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Abstract

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Annelida, traditionally divided into Polychaeta and Clitellata, is an evolutionary ancient and ecologically important group today usually considered to be monophyletic. However, there is a long debate regarding the in-group relationships as well as the direction of evolutionary changes within the group. This debate is correlated to the extraordinary evolutionary diversity of this group. Although annelids may generally be characterised as organisms with multiple repetitions of identically organised segments and usually bearing certain other characters such as a collagenous cuticle, chitinous chaetae or nuchal organs, none of these are present in every subgroup. This is even true for the annelid key character, segmentation. The first morphology-based cladistic analyses of polychaetes showed Polychaeta and Clitellata as sister groups. The former were divided into Scolecida and Palpata comprising Aciculata and Canalipalpata. This systematisation definitely replaced the old concept of dividing polychaetes into Errantia and Sedentaria, whereas the group Archiannelida had already been abandoned. The main critics came from a contradicting hypothesis relying on scenario based on plausibility considerations regarding Clitellata as highly derived annelids nesting within polychaetes and rendering the latter paraphyletic. In this hypothesis the absences of typical polychaete characters were regarded as losses rather than as primary absences. However, to date attempts to unambiguously identify the sister group of Clitellata on the basis of morphological characters have failed. Thus, two hypotheses on the last common annelid ancestor have been put forward either being an oligochaete-like burrowing animal or a parapodia-bearing epibenthic worm. These attempts to understand the major transitions in annelid evolution are reviewed and discussed in the light of new morphological evidence such as photoreceptor cell and eye evolution as well as the evolution of the nervous system and musculature. We also discuss the plausibility of these scenarios with regard to recent advances in molecular phylogenetic analyses.

Keywords polychaetes, oligochaetes, Clitellata, Sedentaria, Errantia, ground pattern, morphology

Introduction

Annelida, traditionally divided into Polychaeta and Clitellata (Rouse and Fauchald, 1995, 1998; Bartolomaeus et al., 2005), is an evolutionary ancient and ecologically important group comprising approximately 16,500 species occurring in marine, limnetic and terrestrial habitats (Struck, 2011; Struck et al., 2011). Their biological importance relies not only on the comparatively high number of species but also on their often high abundance. Although some species can be found in the plankton throughout their entire life span, annelids usually constitute a significant part of the endo- and epibenthos where they occupy almost every existing ecological niche in the marine environment. They occur from the deep sea to the supralittoral zones of sandy beaches. However, the vast majority of the limnetic and terrestrial species belong to only one clade, called Clitellata, the members of which show specific adaptations to terrestrial life (e.g. Purschke, 1999, 2002). Obviously due to subsequent adaptive radiations, this broad ecological range occupied by annelids resulted in a high morphological diversity (fig. 1A-L).

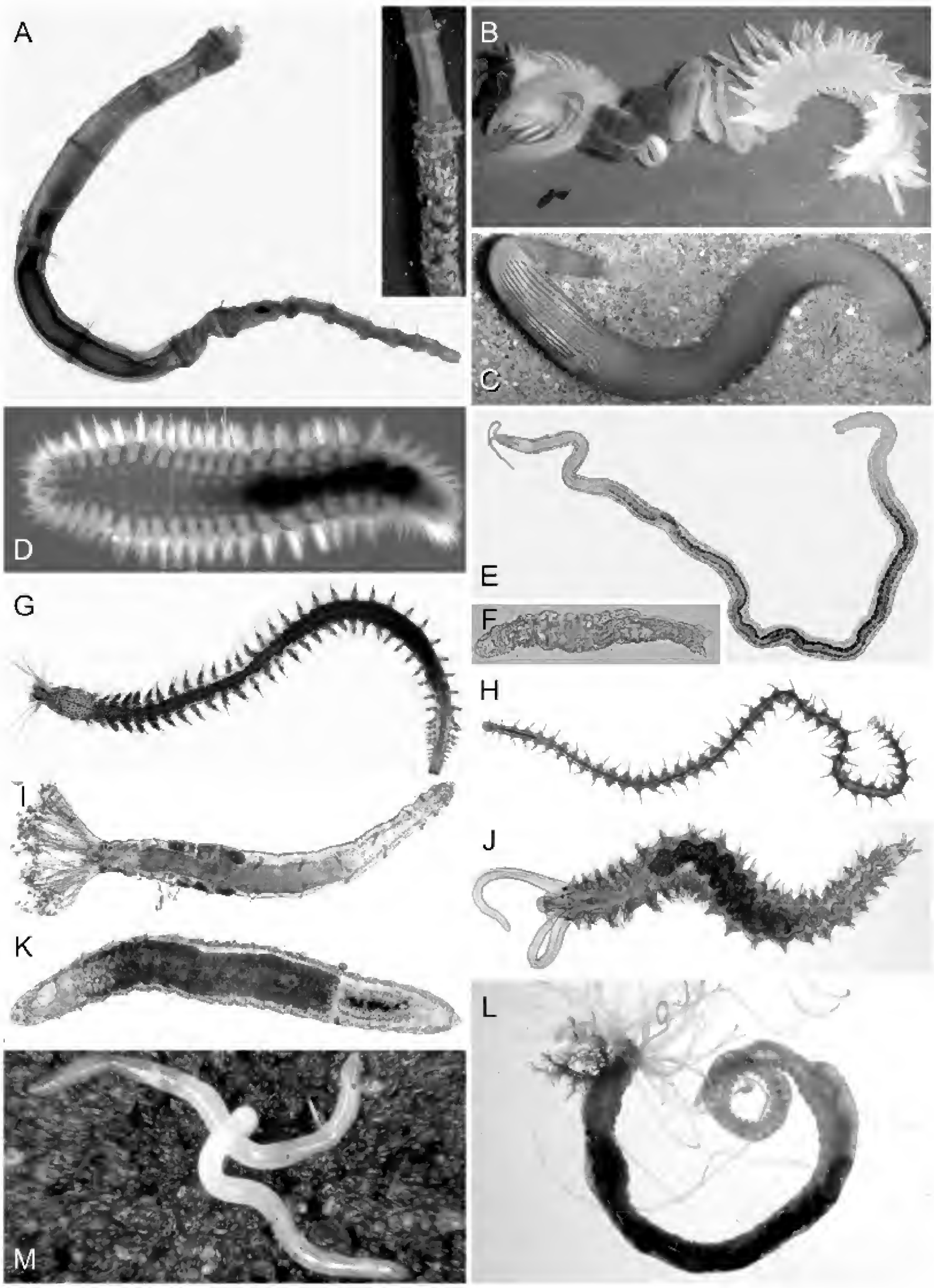

Figure 1. Examples of annelid diversity. A-D. Members of the basal radiation; A. *Owenia fusiformis*, Oweniidae, length about 100 mm, Inset: part of the tube. B. *Chaetopterus variopedatus*, Chaetopteridae, length about 250 mm. C. *Sipunculus nudus*, Sipuncula, length about 350 mm. D. *Eurythoe complanata*, Amphinomidae, length about 140 mm. E-F. Former Archiannelida; E: *Protodriloides chaetifer*, Protodrilida, length about 13 mm; F. *Diurodrilus subterraneus*, length about 440 μm. G-H. Errantia; G. *Platynereis dumerilii*, Nereididae, length about 100 mm. H. *Microphthalmus similis*, incertae sedis, length about 18 mm. I-M. Sedentaria. I. *Fabricia stellaris*, Sabellidae, length about 4 mm. J. *Pygospio elegans*, Spionidae, length about 25 mm. K. *Ophelia rathkei*, Opheliidae, length about 8 mm. L. *Lanice conchilega*, Terebellidae, juvenile, length up to 300 mm. M. *Enchytraeus* sp. Clitellata, length about 15 mm. Originals B, C, D: W. Westheide, Osnabrück.

This diversity is the main reason why the phylogenetic relationships among Annelida are still one of the largest unsolved problems in metazoan phylogeny (Rouse and Fauchald, 1995, 1997; Eibye-Jacobsen and Nielsen, 1996; Westheide, 1997; Westheide et al., 1999; Rouse and Pleijel, 2001, 2003; Purschke, 2002; Bartolomaeus et al., 2005; Struck, 2012). The main problems concern the monophyly of Annelida, the organisation or character composition of the annelid stem species, monophyly versus paraphyly of Polychaeta, the inter-relationships between the various annelid subtaxa as well as the taxon composition of the group (Bartolomaeus et al., 2005; Struck et al., 2011; Struck, 2012). Morphological and molecular evidence increases that taxa which were formerly recognised as separate "phyla" are now regarded as part of the annelid radiation, namely Pogonophora (now Siboglinidae), Echiura, Myzostomida, and Sipuncula (reviewed by Halanych et al., 2002; Struck, 2012; but see Eibye-Jacobsen and Vinther, 2012).

The taxon composition of this presumed monophyletic group Annelida including these former "phyla" is crucial for reconstructing the characters of the annelid stem species or its last common ancestor (Purschke, 2002). As a result of the controversial hypotheses on the taxon composition and phylogeny of Annelida, two hypotheses regarding the last common ancestor have been put forward: either an oligochaete-like burrowing animal, or a parapodia-bearing epibenthic worm. Consequently polychaetes may be monophyletic or paraphyletic (see Bartolomaeus et al., 2005; Struck, 2011). Irrespective of the taxa included, the state of almost every character considered varies greatly among annelids making ground pattern reconstruction a difficult task. Although there is general agreement that Annelida are organisms with a multiple repetition of identically organised segments (Bartolomaeus et al., 2005; Struck, 2011; Hannibal and Patel, 2013), there are certain taxa in which even this so-called key-character is virtually absent: e.g., Echiura, Sipuncula, *Diurodrilus* (Purschke et al., 2000; Wanninger et al., 2005; Worsaae and Rouse, 2008; Nielsen, 2012; Golombek et al., 2013). The number of segments varies between species and may comprise between only 6 or fewer (e. g. *Parapodrilus psammophilus* Westheide, 1965) to more than 1,000 segments (e. g. *Eunice aphroditois* (Pallas, 1788)) resulting in body lengths varying from less than 600 μm to about 6 m (see Paxton, 2000). Presence of segmentally arranged chitinous chaetae is another key-character of annelids (Hausen, 2005a). However, the pleisomorphic condition regarding shape and structure of these chaetae and whether these chaetae were primarily situated in lobe-like appendages, the parapodia, is also a matter of discussion (Rouse and Fauchald, 1997; Bartolomaeus et al., 2005; Struck, 2011). Also, some taxa lack chaetae in all stages of their life cycle (e.g., Polygordiidae; see Ramey et al., 2012).

The aims of the present paper are (1) to briefly review the systematics of annelids, (2) to discuss morphological characters presumably important for the reconstruction of the ground pattern, (3) to elucidate the question of paraphyly of polychaetes, and (4) to identify directions of future research in annelid morphology and phylogeny. Finally, all these are discussed in the light of current molecular phylogenetic analyses of Annelida.

Annelid Systematics

Since the first phylogenetic analyses of molecular and morphological datasets, approximately 20 years ago (Rouse and Fauchald, 1997; McHugh, 1997), systematics of Annelida has been undergoing major reassessments after a period of relative stability. Although a detailed historical review of traditional annelid systematisation can be found in Struck (2012), some highlights are briefly summarised. Annelida as a separate group was first recognised by Lamarck (1802) and included polychaetes, earthworms and echiurans. Audouin & Milne Edwards (1834) divided Annelida into annélides errantes, annélides tubicoles (ou sédentaires), annélides terricoles (= Capitellida + oligochaetes), and annélides soucieuses (= Hirudinea). Errantia included the more vagile forms and Sedentaria the more or less sessile, often microphagous annelid groups. In this concept Annelida obviously was not divided into Polychaeta and Clitellata (or Oligochaeta). The division of Annelida into Polychaeta and Oligochaeta goes back to Grube (1850), retaining the division of polychaetes into two major groups which he called Rapacia and Limivora. This classificatory concept of subdividing polychaetes into Errantia and Sedentaria has been widely accepted and was in use with some modifications for more than 100 years (e. g., Hartmann-Schröder, 1971). A third major annelid group, called Archiannelida, comprising several groups of seemingly simply organised, small annelids was introduced later by Hatschek (1878, 1893). This grouping mirrors the view that "simple equals primitive" (e. g. Jamieson, 1992; but see Hughes et al., 2013).

Archiannelids show an apparently simple organisation and may retain characters otherwise typical for annelid larvae such as ciliary bands used for locomotion (Figs 1E, F, 6C). Their segmentation is often hardly recognisable and many species possess neither chaetae nor parapodia. Most archiannelid species are members of the meiofauna of marine sediments (interstitial annelids). Errantia may be morphologically characterised by well-developed parapodia often equipped with dorsal and ventral cirri, prostomial antennae and palps, usually with a high number of homonymous segments, one or several pairs of tentacular (peristomial) cirri, a pair of pygidial cirri, and adult individuals usually with one or two pairs of pigmented, multicellular eyes (fig. 1G, H). Often three subgroups are distinguished: Amphinomida, Eunicida, and Phyllodocida. By contrast, Sedentaria are much more diverse (fig. 1I-L) and may be characterised by more or less simple or even lacking parapodia, usually without dorsal and ventral cirri, typically with hooked chaetae (uncini); palps and pygidial cirri are either absent or present whereas antennae and peristomial appendages are always lacking. Pigmented adult eyes are usually of the larval type in this group; i.e. bicellular only, comprising one photoreceptor and one pigment cell. These polychaetes often have fewer segments than errant polychaetes and the body may be divided into different regions (Hartmann-Schröder, 1971; Fauchald, 1977; Bartolomaeus et al., 2005; Purschke et al., 2006; Suschenko and Purschke, 2009). With respect to the characters mentioned above Clitellata show simple chaetae and lack parapodia as well as

any appendage on the prostomium, peristomium and pygidium (fig. 1M). On the other hand, clitellates show an exclusive combination of numerous characters such as the clitellum, hermaphroditism, a specific type of spermatozoon, a dorsal pharynx, a specific type of photoreceptor cell (= phaosome), and a posteriorly dislocated brain, supporting their monophyly (Purschke, 2002; Bartolomaeus et al., 2005).

Dales (1962, 1963) was among the first to question this traditional concept (Dales, 1963, p. 64): "The polychaetes are, indeed, most usually divided into two subclasses, the Errantia and the Sedentaria. This division is not a natural one, however, and does not reflect the way in which these worms, have evolved ...". He proposed a classification based on analysing the distribution of characters such as buccal organs and nephridia. A similar approach has been adopted by Storch (1968) using muscular systems as the most important characters. Although neither classification gained general acceptance, polychaete subtaxa usually were placed at equal rank in the following years, retaining Polychaeta and Clitellata as highest ranked taxa. Fauchald (1977), obviously inspired by Clark's (e.g. 1964) ideas of an earthworm-like annelid ancestor, placed the oligochaete-like forms at the base of the polychaetes. Although listed without any interrelationships specified, Fauchald (1977, p. 7) stated: "the sequence of families indicates an increasing morphological distance from the ancestral polychaete" implying that the groups listed first were presumably closer to the annelid stem species than the following ones. In parallel, Archiannelida was recognised as an artificial, presumably polyphyletic assemblage of interstitial annelids primarily adapted to life in the mesopsammon (e. g., Hermans, 1969; Fauchald, 1974; Westheide, 1985, 1987).

Westheide (1997) questioned the sister group relationship of Polychaeta and Clitellata and considered Polychaeta paraphyletic and Clitellata being sister to an unknown polychaete taxon. However, in the same year the first hypothesis based on cladistic analyses was published (fig. 2A; Rouse and Fauchald, 1997). This phylogenetic hypothesis was widely accepted in a comparatively short period of time, introduced to many textbooks and is still in use – of course with some modifications (see e. g. Rouse and Pleijel, 2001, 2003). These first morphological-based cladistic analyses of polychaetes showed Polychaeta and Clitellata as sister groups contradicting the hypothesis of a paraphyletic Polychaeta (Westheide, 1997). In the hypothesis of Rouse and Fauchald (1997) Polychaeta were divided into Scolecida and Palpata. Scolecida comprised the more or less oligochaete-like appendage-less polychaetes, whereas Palpata contained all palp-bearing polychaetes. Palpata were subdivided into Aciculata and Canalipalpata. Irrespective of the fact that Aciculata and Errantia comprise the same subtaxa, this systematisation replaced the old concept dividing polychaetes into Errantia and Sedentaria. Interestingly, as already suggested by Bartolomaeus (1995, 1998) and by the hypothesis of Rouse & Fauchald (1997), Pogonophora forms a polychaete in-group (which was subsequently called Siboglinidae), but Echiura and Sipuncula were still excluded from Annelida based mainly on the lack of annelid key characters such as segmentation and chaetae.

The main criticism on the hypothesis of Rouse & Fauchald (1997) came from a contradicting hypothesis which regarded Clitellata as highly derived annelids forming a polychaete in-group and rendering the latter paraphyletic (Purschke, 1997, 1999, 2000, 2002, 2003; Westheide, 1997; Westheide et al., 1999; Bartolomaeus et al., 2005). Although to date all attempts have failed to unambiguously identify the sister group of Clitellata, in this hypothesis the absence of typical polychaete characters in Clitellata and Echiura is regarded as losses rather than as primary absences (Purschke, 1997, 1999; Purschke et al., 2000).

It is suggested that cladistic analyses using morphological data may fail to recognise absent characters as losses rather than as primary absences (Purschke et al., 2000; Bleidorn, 2007; see Fitzhugh, 2008). Thus, the sister-group relationship Polychaeta-Clitellata as found in Rouse and Fauchald (1997) may have been biased by the misinterpretation of a number of convergently lost characters. Likewise the highly derived nature of several characters of Clitellata related to their adaptations to terrestrial life was not recognised. In contrast, according to Rouse & Fauchald (1997) Clitellata should more or less resemble the annelid stem species. For the same reasons exclusion of Echiura and Sipuncula from Annelida might represent an analytical artifact. Careful analyses of the development of the latter taxa provided evidence for a reduced rather than absent segmentation (Hessling, 2002; Hessling and Westheide, 2002; Tzetlin and Purschke, 2006; Kristof et al., 2008).

This morphology based cladistic hypothesis was never supported by molecular phylogenetic analyses, but if included Clitellata usually appeared as a polychaete in-group (e.g., McHugh, 1997; Bleidorn et al., 2003; Rousset et al., 2007; Zrzavy et al., 2009; Struck et al., 2007, 2008, 2011; Weigert et al., 2014). In addition, monophyly of the basal group Scolecida was never recovered by molecular analyses. Whereas the first molecular analyses suffered from low or lack of support for deep nodes in the annelid tree, current analyses now relying on phylogenomic datasets based on hundreds of genes show high support for even deep nodes in the annelid tree (Struck et al., 2011; Weigert et al., 2014; but see Kvist and Siddall, 2013). These analyses recover a basal grade comprising several enigmatic taxa such as Chaetopteridae, Oweniidae, Magelonidae as well as Sipuncula and Amphinomidae (Weigert et al., 2014). The vast majority of annelid taxa form a monophyletic group named Pleistoannelida (Struck, 2011), with Errantia and Sedentaria being the highest ranked sister groups, the latter including Clitellata (fig. 2B). However, it should be noted here that the taxon composition and definition of both Errantia and Sedentaria is slightly different from the traditional concepts (Struck et al., 2011; Struck, 2012; Weigert et al., 2014). Interestingly, a comparison of trees obtained from phylogenomic analyses to those obtained using morphological data show that the major difference is the placement of the root of the annelid tree either within the former Palpata or close to Clitellata, respectively (Struck, 2012).

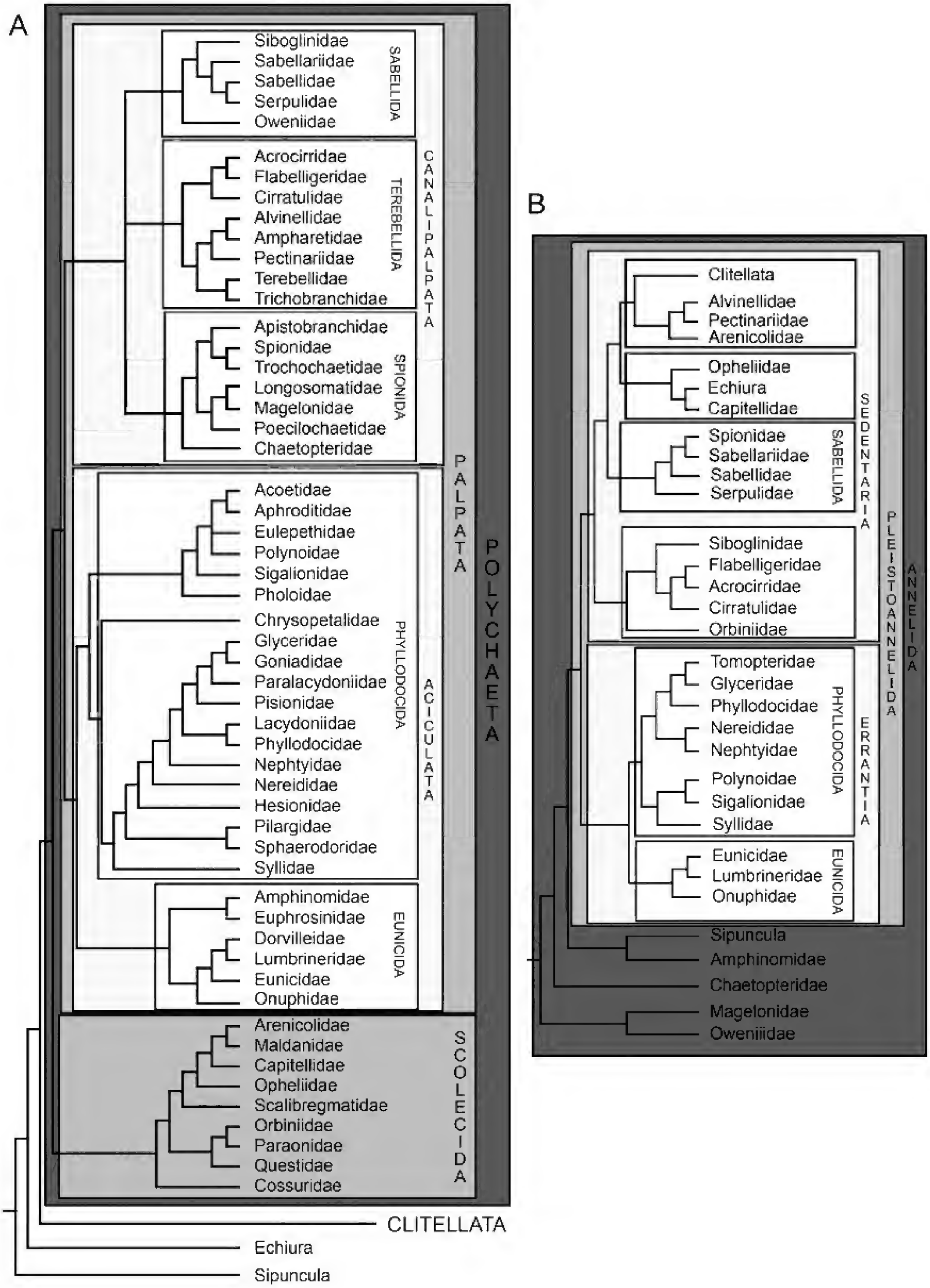

Figure 2. Phylogenetic hypotheses of annelid relationships. A. Cladistic analysis based on morphological data (modified from Rouse and Fauchald 1997). B. Phylogenetic tree based on phylogenomic data (modified after Struck et al. 2011; Weigert et al. 2014).

Morphological characters of Annelida

This conflict on the systematisation of Annelida may lead to differences in the reconstruction of the annelid ground pattern. Despite existence of certain outstanding studies on annelid anatomy, earlier polychaete systematics was largely based on external morphology (reviewed e.g. by Fauchald and Rouse, 1997) and even though some of these studies were extremely comprehensive, additional morphological characters are needed to develop well-founded homology hypotheses (Fauchald and Rouse, 1997; Müller, 2006). Recently, fine structural investigations (cLSM, TEM, SEM) as well as developmental ones have provided such data and may provide better evidence for homology considerations (e.g., Orrhage and Müller, 2005; Müller, 2006; Hunnekuhl et al., 2009; Suschenko and Purschke, 2009; Wilkens and Purschke, 2009a, b; Filippova et al., 2010; Döring et al., 2013; Lehmacher et al., 2014; see also Fauchald, 1977; Fauchald and Rouse, 1997). These studies have mainly focussed on the muscular system, nervous system and sensory organs. Another source of data is the determination of the so-called molecular fingerprint (gene expression patterns) of cell types for homology assessments (e. g. Arendt, 2008; Arendt et al., 2009; Döring et al., 2013).

Given the two main opposing morphology-based phylogenetic hypotheses discussed above it is surprising that the differences in the ground pattern of the annelid stem species are smaller than might be expected. According to Fauchald (1974) the ancestral annelid resembled a polychaete and was characterised by complete septation, distinct segments, chaetae and low parapodial folds, anterior end without appendages and a burrowing lifestyle. The stem species was a marine, gonochoristic, broadcast spawner with a planktotrophic larva. This hypothesis was only slightly changed after Rouse and Fauchald's (1997) cladistic analysis: according to this hypothesis the last common ancestor of Annelida was homonomously segmented, the longitudinal musculature not forming a continuous layer but consisted of 4-5 longitudinal bands, the gut as a straight tube with dorsolateral folds in the foregut, chaetae all simple capillaries, the prostomium distinctly set off but with no appendages, nuchal organs, and internal supporting chaetae and parapodia absent. The annelid stem species after Weigert et al. (2014) was homonomously segmented, with longitudinal muscle bands, the gut forming a straight tube with dorsolateral folds in the foregut (microphagous deposit feeder), simple chaetae emerging from parapodia, prostomium and peristomium present with palps, and bicellular eyes present. Thus the main differences are the structure of the prostomium, presence or absence of anterior appendages, the presence of nuchal organs, the nature of the eyes and structure of parapodia. Therefore, these structures and others which have largely been neglected, such as the cuticle and the nervous system, will now be discussed in more detail. Other character complexes will only be mentioned briefly as they have been discussed previously or they will not be discussed as they lack any phylogenetic signal with respect to this question (e.g., Purschke, 2002; Bartolomaeus et al., 2005). These include the mesoderm, the coelom and the nephridia (Rieger and Purschke, 2005; Bartolomaeus and Quast, 2005), pharynx and intestine (Tzetlin and Purschke 2005) as well as the biphasic life cycle (Rieger, 1994; Rieger and Purschke, 2005; Nielsen, 2012).

Segmentation

The annelid body generally consists of a small presegmental region, the prostomium, a segmented trunk, and a small postsegmental region, the pygidium (fig. 3A-C; see Fauchald and Rouse, 1997; Hutchings and Fauchald, 2000; Rouse and Pleijel, 2001; Purschke, 2002; Bartolomaeus et al., 2005). The prostomium contains the brain (cerebral ganglia) as well as the most important sensory structures. The pygidium bears a terminally or dorsally positioned anus. The mouth is situated ventrally in the first segment, usually called the peristomium. New segments are formed in the posterior growth zone in front of the pygidium. Each segment generally comprises a pair of ganglia in the ventral nerve cord, a pair of coelomic cavities, a pair of metanephridia, and paired ventral and dorsal groups of chaetae (see Purschke, 2002; Bartolomaeus et al,. 2005). The leeches show obvious signs of reduced but still recognisable segmentation: for instance, the ventral nerve cord clearly allows the number of segments comprising the body to be determined (Purschke et al., 1993).

Most annelid groups regarded as lacking segmentation such as Siboglinidae, Echiura, Sipuncula and *Diurodrilus* generally show signs of suppression or reduction of segmentation (fig. 1C, F). Among these Siboglinidae are the most obviously segmented, when the often-missing posterior part of the body was found (Webb, 1964; Southward, 1988; Southward et al., 2005). Only subtle traces of segmentation have been found in developmental stages of echiuroids and sipunculans whereas in adults all signs of segmentation are absent (Hessling and Westheide, 2002; Hessling, 2003; Wanninger et al., 2005; Kristof et al., 2008). Species of *Diurodrilus*, a group of small interstitial animals, do not exhibit any signs of segmentation even in the nervous system (Worsaae and Rouse, 2008): However, molecular phylogenetic data and other morphological characters clearly support the inclusion of this taxon within Annelida (see Golombek et al., 2013).

Whereas formerly segmentation in arthropods and annelids has generally been assumed to be a synapomorphic character, the early molecular phylogenetic analyses raised doubts regarding a single evolutionary origin of segmentation in these taxa (for summary see Dordel et al., 2010). Increasing molecular developmental data demonstrates evidence for a convergent origin of segmentation (see Shankland and Seaver, 2000; Seaver, 2003; De Rosa et al., 2005; Seaver et al., 2012). However, others have proposed that the last common ancestor of Bilateria was already segmented (de Robertis et al., 2008; Couso, 2009; Chesebro et al., 2013).

Cuticle

Without exception a collagenous cuticle completely covers the annelid epidermis (Storch, 1988; Gardiner, 1992; Hausen, 2005b). The cuticle is composed of an amorphous or filamentous matrix that usually houses layers of parallel collagen fibres which are oriented perpendicularly between the layers (fig. 4A-E). Presumably the matrix is composed of different

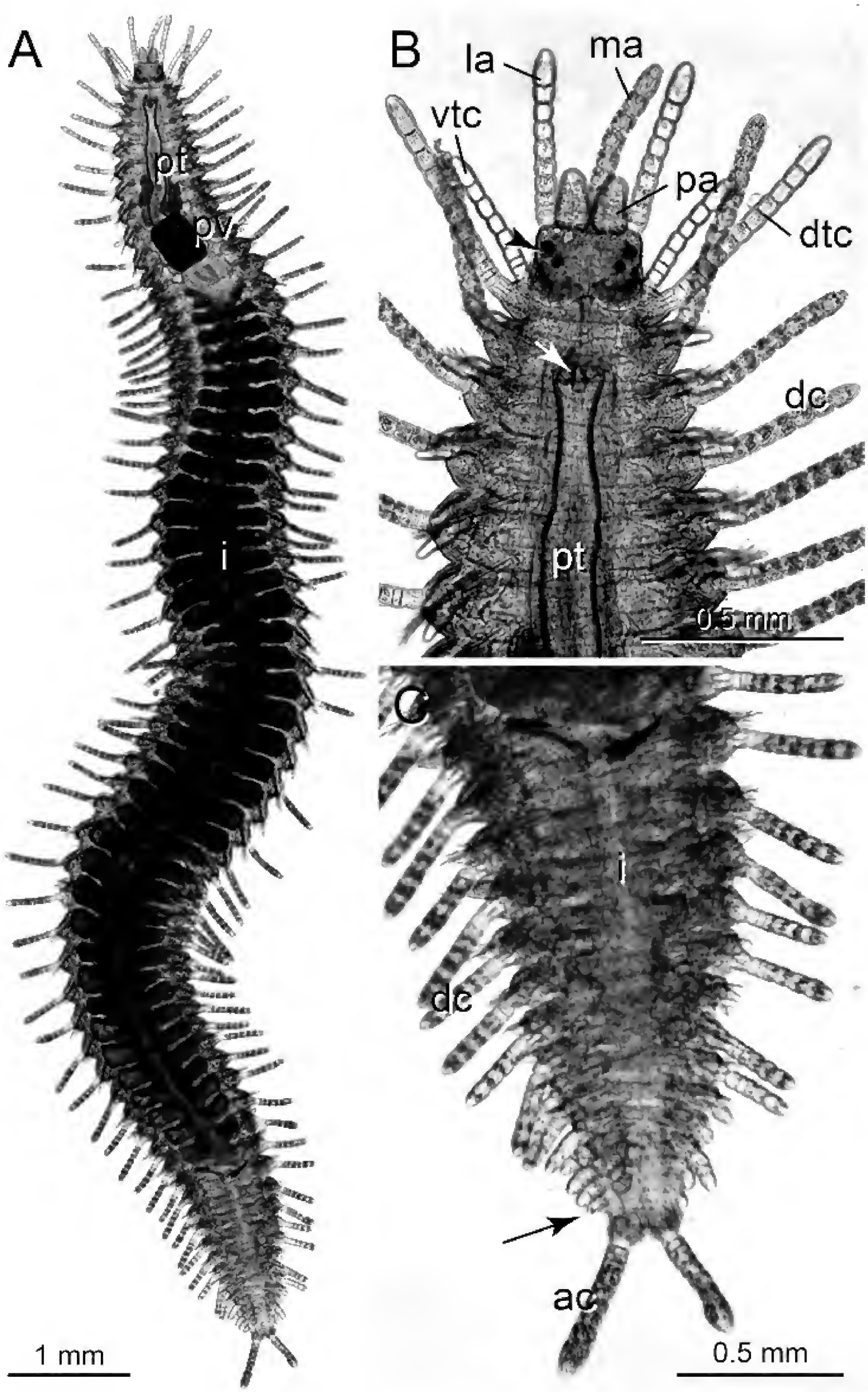

Figure 3. General organization of an annelid exemplified with *Trypanosyllis coeliaca* (Errantia, Syllidae). A. Entire animal. B. Enlargement of head region; arrowhead: pigmented eyes; arrow: pharynx tooth. C. Posterior end with growth zone (arrow). - ac = anal cirrus, dc = dorsal cirrus, dtc = dorsal tentacle cirrus, i = intestine, la = lateral antenna, ma = median antenna, pa = palp, pt = pharyngeal tube, pv = proventricle. Micrographs of living specimen.

mucopolysaccharides and hyaluronic acid (Hausen, 2005b). The uppermost part is usually devoid of collagen fibres and is called an epicuticle. The cuticle is traversed by microvilli extending above the surface and either forms isolated epicuticular projections or multiple tips. This uppermost part is covered by a glycocalix. A cuticle exhibiting these characteristics is found in all major annelid clades including Sipuncula, although considerable variation occurs (fig. 4A-E). The cuticle may vary in thickness, number of microvilli, or development of collagen fibres. Especially in larvae and adults of small or interstitial species the collagen fibres appear to be less developed, sometimes more irregularly arranged or even absent. In these cases the cuticle more or less resembles the egg envelope from which it originates (Eckelbarger, 1978). However, there are other examples of polychaetes with less well-developed layers of collagen fibres among polychaetes such as found in chaetopterids, oweniids, magelonids, apistobranchids and psammodrilids (fig. 4 D, E; Kristensen and Nørrevang, 1982, Hausen, 2001, 2005b, 2007). Absence of collagen fibres in the cuticle is thus observed in most groups belonging to the basal radiation according to Weigert et al. (2014) indicating that the presence of grids of collagen fibres probably is an autapomorphy of the clade comprising Amphinomida , Sipuncula and Pleistoannelida. Thus, the relevance of the cuticle as a phylogenetic important character and as a possible autapomorphy of the entire group has so far been underestimated (Purschke, 2002).

Chaetae and Parapodia

Chaetae are generally regarded as the most characteristic and important taxonomic feature of Annelida. They constitute the most thoroughly studied annelid structures (for references see Rouse and Fauchald, 1995, 1997; Westheide, 1997; Rouse and Pleijel, 2001; Hausen, 2005a). Chaetae have various functions and may aid in locomotion on the substrate, anchoring the body inside the tubes, protecting and defending the body, supporting parapodia, etc. Accordingly they show an extraordinary structural diversity and often exhibit species-specific characters (Hausen, 2005a). On the basis of light microscope investigations several types of chaetae are distinguished (Rouse, 2000; Rouse and Pleijel, 2001). The most common type represented by thin tapering cylinders is the simple or capillary chaetae, which may be smooth or have various additional substructures and ornamentations (fig. 5A, F). Capillaries are often regarded as representing the plesiomorphic type (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001; Struck et al., 2011).

Irrespective of their external diversity, the formation and ultrastructure of chaetae appears very uniform: Each chaeta is made up of many longitudinal tubules consisting of chitin cross-linked by proteins situated in epidermal follicles. Chaetae are formed by a single cell called a chaetoblast and its dynamic microvilli are responsible for the variations in form and diameter of tubules as well as the external structure of the

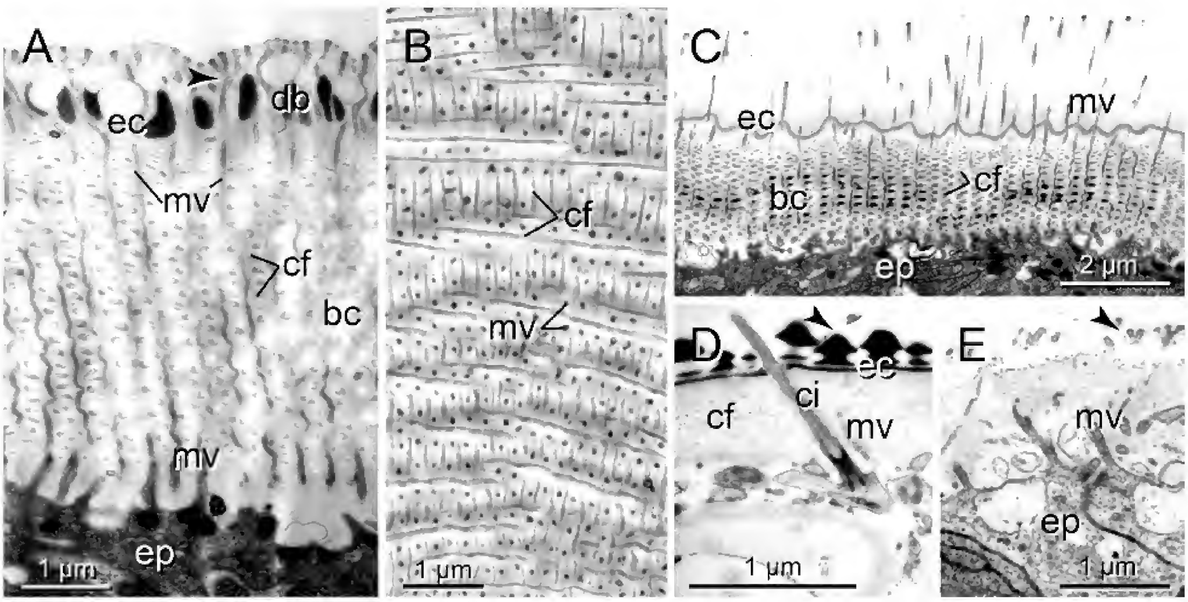

Figure 4. Cuticle ultrastructure of annelids. A-B. *Eurythoe complanata* (Amphinomidae). A. Cross section of cuticle on the trunk. Cuticle made up of layers of parallel collagen fibres (cf) traversed by microvilli (mv), which branch apically above the epicuticle (ec, arrowhead), epicuticle (ec) with dense bodies (db). B. Tangential section showing arrangement of collagen fibres and microvilli. C. *Polygordius appendiculatus* (Polygordiidae). Microvilli extend far above epicuticle (ec). D. *Sphaerodoropsis minuta* (Sphaerodoridae). Cuticle with irregularly arranged hardly visible collagen fibres (cf), covered by dark disk-like structures (arrowhead); cuticle traversed by cilium (ci) of receptor cell. E. *Ophiodromus pallidus* (Hesionidae). Cuticle without collagen fibres, microvilli branch above cuticle (arrowhead). – bc = basal cuticle, cf = collagen fibre, ci = cilium, db = dense body, ec = epicuticle, mv = micovillus. TEM micrographs.

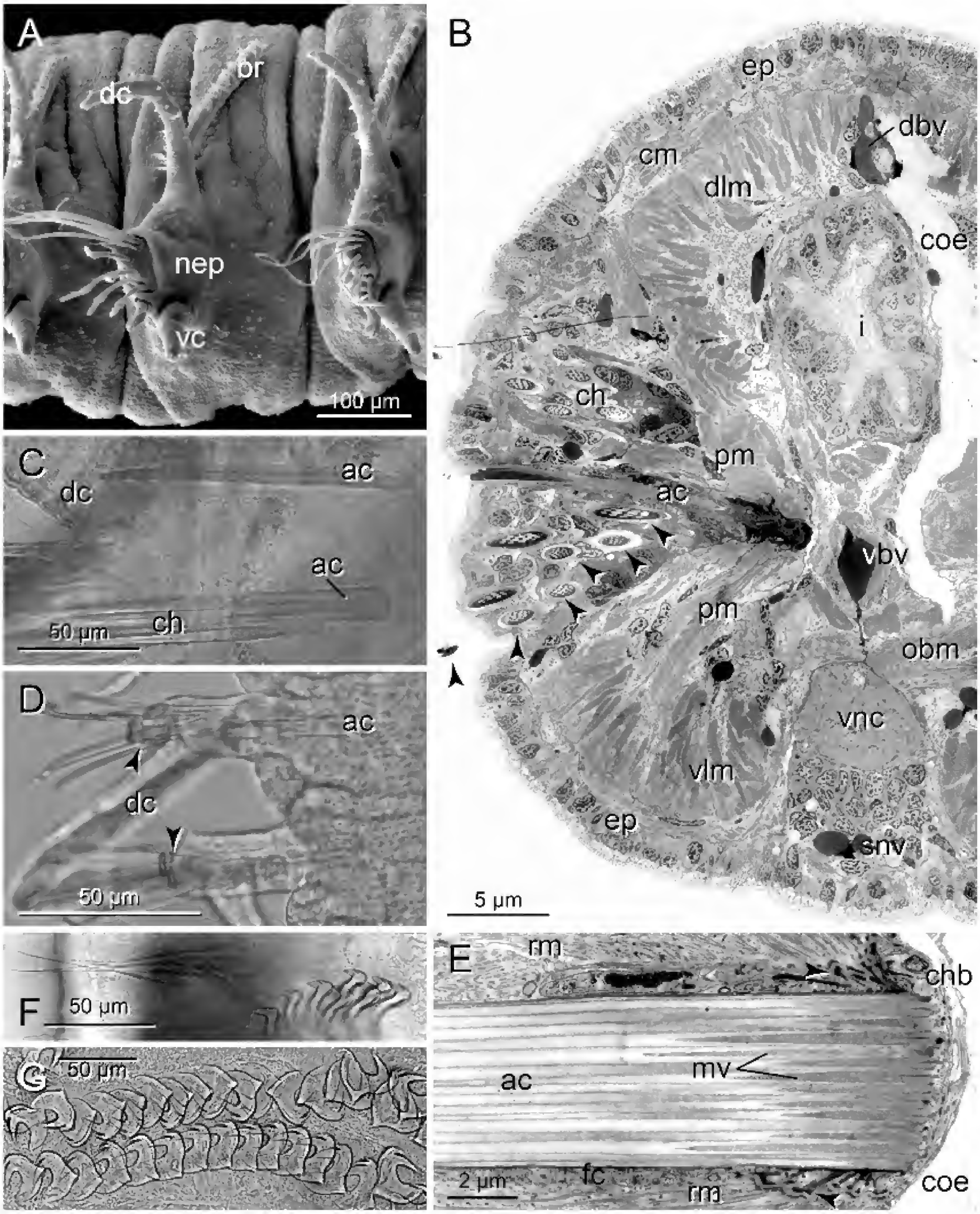

Figure 5. Parapodia and chaetae. A. *Eunice pennata* (Eunicidae); parapodium comprised of dorsal cirrus (dc), neuropodium (nep), ventral cirrus (vc) and branchia (br); acicula invisible. SEM micrograph. B. *Scoloplos armiger* (Orbiniidae). Cross section showing parapodium with supportive chaetae and chaetal sac; arrowheads point to sectioned chaetae. TEM micrograph. C. *Syllidia armata* (Hesionidae); notopodium restricted to dorsal cirrus and acicula (ac). D. *Streptosyllis websteri* (Syllidae). Aciculae (ac) extending outside parapodial lobe (arraowheads). E. *Sphaerodopsis minuta* (Sphaerodoridae). Acicula with chaetoblast (chb); arrowheads point to junctional complexes. F. *Fabricia stellaris* (Sabellidae). Parapodium of thorax with capillaries and uncini. G. *Lanice conchilega* (Terebellidae). Uncini. - ac = acicula, br = branchia, ch = chaeta, cm = circular muscle, coe = coelom, dc = dorsal cirrus, dlm = dorsal longitudinal muscle, ep = epidermis, dbv = dorsal blood vessel, fc = follicle cell, i = intestine, mv = microvillus, nep = neuropodium, obm = oblique muscle, pm = protractor muscle, rm = retractror muscle, snv = subneural blood vessel, vbv = ventral blood vessel, vc = ventral cirrus, vlm = ventral longitudinal muscle, vnc = ventral nerve cord. C, D, F, G: micrographs from living specimens, slightly squeezed.

chaetae (fig. 5B, E; Purschke, 2002; Hausen, 2005a). As a rule these tubules show diminishing diameters from the centre to the periphery. The chaetoblast forms the base of an epidermal follicle lined by follicle and typical epidermal supportive cells; the follicle cells lacking a cuticle (fig. 5E). Follicle cells and the chaetoblast also function in mechanical coupling of the chaeta due to prominent myoepithelial junctions, extensive intermediate filaments and apical hemidesmosomes (fig. 5E; Specht, 1988; Hausen, 2005a). Depending on the arrangement and function chaetae may be individually moveable or form functional groups situated in a common chaetal sac.

Among the various types of chaetae, a few have been used to define higher-level in-group relationships including aciculae (fig. 5B-D, E), uncini, hooks (fig. 5F, G) and paleae (Bartolomaeus et al., 2005; Hausen, 2005a). The former are supportive chaetae in parapodia, deeply anchored in the tissues and normally not exposed to the exterior although in certain taxa they can protrude slightly (fig. 5B-D; Fauchald and Rouse, 1997; Rouse and Pleijel, 2001; Hausen, 2005a). Aciculae are not formed in the same chaetal sacs as the other chaetae in the same fascicle (fig. 5B). These chaetae function as skeleton for the entire parapodial lobes. Aciculae have been regarded as being homologous in Amphinomida, Eunicida and Phyllodocida and represent the most important synapomorphic character uniting these groups as Aciculata (Rouse and Fauchald, 1997). However, supporting chaetae are also present in other polychaete groups such as Chaetopteridae, Orbiniidae, Apistobranchidae, Psammodrilidae and Myzostomida (Hausen, 2005a). Nevertheless, there is a still ongoing debate as to whether these supportive chaetae are homologous or convergent structures (Fauchald and Rouse, 1997; Rouse and Pleijel, 2001; Hausen, 2005a; Hoffmann and Hausen, 2007; Struck, 2011; Struck et al., 2011; Eibye-Jacobsen and Vinther, 2012). As stated by, e.g. Rouse and Pleijel (2001, p.23): "aciculae are formed exactly in the same manner as the projecting chaetae", this question can hardly be solved by morphological studies alone.

Other types of chaetae, which have received much attention, are the hooks and uncini. Such chaetae are usually present in tube-building polychaetes (fig. 5F, G). Due to a high degree of similarity in structure and in their process of formation they have been regarded to be homologous across polychaetes, potentially supporting a clade uniting those taxa bearing this character (Bartolomaeus et al., 2005; Hausen, 2005a). An opposite view was taken by Rouse and Fauchald (1997) who, based on their cladistic analyses, regarded uncini as being evolved independently in several lineages. Recent phylogenomic studies (Struck et al., 2011; Weigert et al., 2014) have not helped resolving this question, since taxa such as Oweniidae and Chaetopteridae, either possessing hooks or uncini, are part of the basal annelid radiation. Struck et al. (2011) indicated these chaetae as a possible apomorphy for Sedentaria and thus they also hypothesised convergent evolution of this type of chaetae. However, it has not been ruled out, whether these highly specific chaetae were also present in the annelid stem species and have been lost repeatedly. Parsimony-based ancestral character state reconstructions point to that direction.

Appendages of the prostomium - antennae and palps

Head appendages include antennae, palps, peristomial cirri and in more cephalised polychaetes also cirri of anterior segments (Rouse and Pleijel, 2001) (Figs 3A, B, 6A-E, 10G, I). Among these, only antennae and palps appear phylogenetically informative for the deep nodes since peristomial cirri are restricted to a few taxa within Eunicida and Phyllodocida.

Antennae are prostomial sensory appendages usually present in representatives of Amphinomida, Eunicida and Phyllodocida (Rouse and Pleijel, 2001; Purschke, 2002). There may be a pair of lateral antennae and an unpaired median antenna resulting in between 0 and 3 appendages. Generally they are more or less digitiform (Figs 6A, B, 7A, B) ranging from smooth to articulated and are divided into a basal ceratophore and a ceratostyle. Due to their corresponding innervation pattern they have been regarded as homologous throughout annelids (fig. 11F; Orrhage and Müller, 2005). Antennae are innervated from the dorsal commissure of the dorsal root of the circumoesophageal connectives. Each lateral antenna receives one nerve whereas in the median antenna there are two nerves separated by a muscle band attaching to its base. Whether this also applies for the unpaired median appendages (antennae or occipital tentacles) present in certain Spionidae and Paraonidae is a matter of discussion (see Orrhage, 1966; Fauchald and Rouse, 1997; Rouse and Pleijel, 2001; Orrhage and Müller, 2005). However, their innervation pattern is the same as in the median antenna of the errant forms and their homology would imply that they represent the plesiomorphic condition and that repeated losses have occurred in sedentary polychaetes. Again antennae may then be an autapomorphy of a clade comprising Pleistoannelida, Amphinomida and Sipuncula.

A pair of palps is present in many but not all annelids (Rouse and Pleijel, 2001; Purschke, 2002) (Figs 6A-E, 10G, I). In contrast to antennae, palps exhibit a considerably greater structural diversity. Often two types of palps are distinguished: prostomial (also called sensory or solid) and peristomial (also termed grooved, feeding or hollow) palps (Fauchald and Rouse, 1997; Rouse and Pleijel, 2001; Struck et al., 2011). However, irrespective of these classifications, it must be kept in mind that palps of any kind are sensory but only the so-called sensory palps are solely sensory (Amieva and Reed, 1987; Purschke, 2002, 2005).

Moreover, the terms solid or hollow palps are somewhat misleading, since all palps usually comprise mesodermal tissues at least in the form of musculature and often coelomic cavities as well (Orrhage, 1964, 1974; Gardiner, 1978; Amieva and Reed, 1987; Purschke, 1993). This is also the case for the palpophores of *Nereis* sp. which possesses sensory palps (fig. 6D, E). Irrespective of the presence of coelomic cavities, these mesodermal tissues are separated by a distinct extracellular matrix from the epidermis and nerves (Purschke, 1993). Coelomic cavities forming hollow palps are for example present in Protodrilidae and Saccocirridae (fig. 6C; see Purschke, 1993, Purschke and Jouin-Toulmond, 1994), taxa which have been assigned by Rouse and Fauchald (1997) to belong to Canalipalpata and which lack feeding palps.

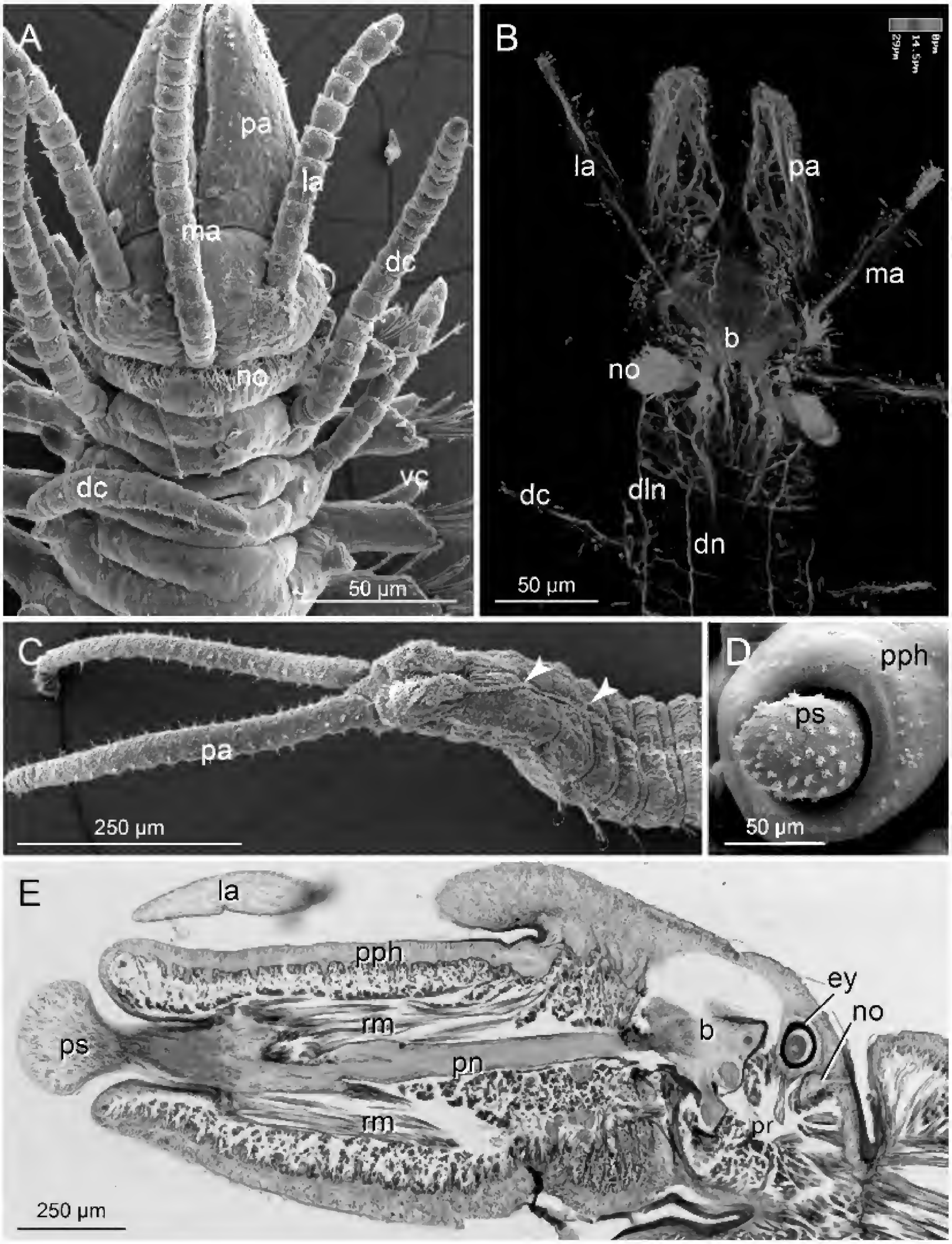

Figure 6. Head appendages and innervation. A. *Syllis* sp. (Syllidae). Anterior end with palps (pa), median (ma) and lateral antennae (la), nuchal organs (no), tentacular cirri on the right broken off. B. *Parapionosyllis labronica* (Syllidae). Dorsal view, nervous system labelled with antibody against acetylated □-tubulin, appendages supplied with prominent nerves, depth coding. C. *Saccocirrus* sp. (Saccocirridae). Ventral view, note ventral ciliated band (arrowheads), palps (pa) supplied with numerous ciliated sensory cells. D, E. *Nereis* sp. (Nereididae). Palp. D. Palp composed of palpophore (pph) and palpostyle (ps) the latter with numerous sensory cilia. E. Longitudinal section showing musculature and coelomic cavity inside palpophore (pph) and connection of palp nerve (pn) with the brain (b). - b = brain, dc = dorsal cirrus, dln = dorsolateral nerve, dn = dorsal nerve, ey = eye, la = lateral antenna, ma = median antenna, no = nuchal organ, pa = palp, pn = palp nerve, pph = palpophore, pr = prostomium, ps = palpostyle, rm = retractor muscle, vc = ventral cirrus. A, C, D: SEM micrographs, Originals S. Raabe & W. Mangerich, Osnabrück; B: cLSM micrograph, original M. Kuper, Osnabrück; E: Azan staining.

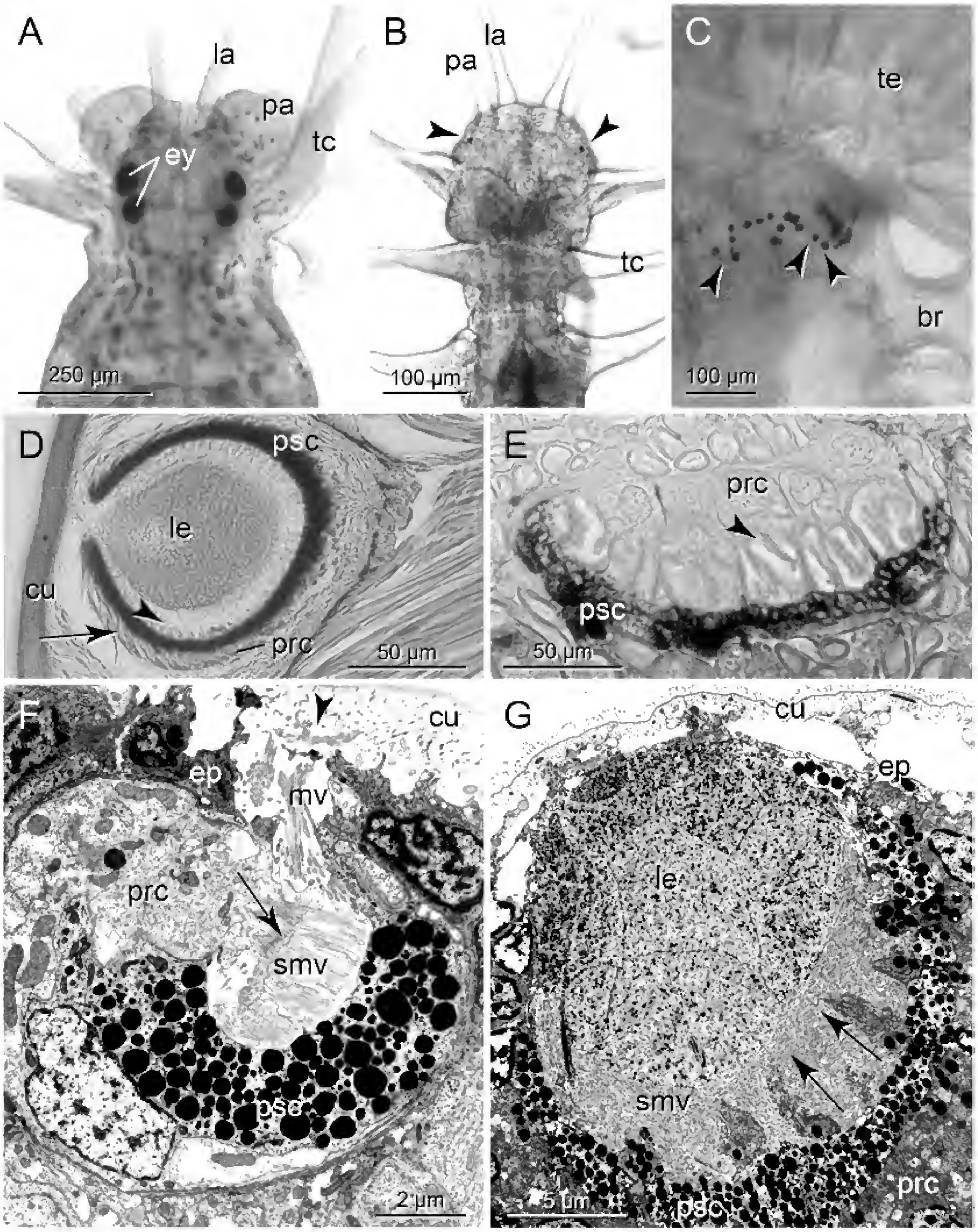

Figure 7. Pigmented eyes. A. *Platynereis dumerilii* (Nereididae). Two pairs of adult eyes (ey) situated on the prostomium. B. *Microphthalmus similis* (Errantia, incertae sedis). Arrowheads point to small prostomial eyes. C. *Nicolea zostericola* (Terebellidae). Numerous small pigmented eyes below tentacular crown (arrowheads). D. *Nereis* sp. (Nereididae). Section showing pigmented eye with lens (le); arrowhead points to zone with rhabdomeres, arrow: marks layer of cell bodies of photoreceptor cells below pigment cell layer (psc). E. *Piscicola geometra* (Clitellata). Pigmented eye with phaosomous photoreceptor cells (prc), arrowhead points to phaosomes. F. *Saccocirrus papillocercus* (Saccocirridae). Small pigmented eye, structurally indistinguishable from larval eye; arrow indicates inverse orientation of photoreceptive structures, eye cup communicates with exterior via small pore (arrowhead). G. *Gyptis propinqua* (Hesionidae). Multicellular-pigmented eye with lens, arrows indicate converse orientation of photoreceptive processes. - br = branchia, cu = cuticle, ep = epidermis, ey = eye, la = lateral antenna, le = lens, pa = palp, prc = photoreceptor cell, psc = pigmented supportive cell, smv = sensory microvilli, tc = tentacular cirri, te = tentacle. A-C: micrographs from living animals; D, E: histological sections, Azan staining; F, G: TEM micrographs.

However, this placement has not been supported by recent molecular phylogenetic investigations and so their systematic position remains unresolved (e. g. Struck et al., 2008; Zrzavý et al., 2009; Golombek et al., 2013). Also *Protodriloides* (Fig 1E), which is regarded as closely related to these taxa, possesses palps without coelomic cavities but with musculature and blood vessels (Purschke, 1993). Moreover, in molecular analyses by Struck et al. (2008) and Zrzavý et al. (2009) Polygordiidae usually fall in the same clade comprising Protodrilidae and Saccocirridae although Polygordiidae may be one of only a few examples for polychaetes with true "solid" palps since their stiff palps lack both musculature and coelomic cavities (Wilkens and Purschke, 2009a). The same applies to the appendages of Sphaerodoridae which are devoid of musculature and are stiff as well (Filippova et al., 2010). Previously it has been assumed that palps of all errant taxa lack musculature, coelomic cavities and blood vessels (Purschke, 2005). But analyses of Syllidae and Dorvilleidae as well as of Nerillidae revealed the presence of well-developed musculature in the palps of errant polychaetes (Filippova et al., 2006, 2010; Müller and Worsaae, 2006). A highly developed muscular system is also present in, e.g., the palps of adults in Magelonidae (see Filippova et al., 2005), which are placed in the basal part of the annelid tree in a recent phylogenomic analysis (Weigert et al., 2014).

Irrespective of whether adult palps are prostomial or peristomial, they are regarded as homologous due to their corresponding innervation from the dorsal and ventral roots of the circumoesophageal connectives (Fauchald and Rouse, 1997; Rouse and Pleijel, 2001; Orrhage and Müller, 2005). There are up to 12 palp nerve roots, which can be homologised due to their positions and relations to other nervous elements (Figs 6E, 11F; Orrhage and Müller, 2005). However, no annelid taxon studied to date exhibits all these roots and so far a ground pattern has not been reconstructed. Usually there are two main palp nerve roots (comparatively thick nerves

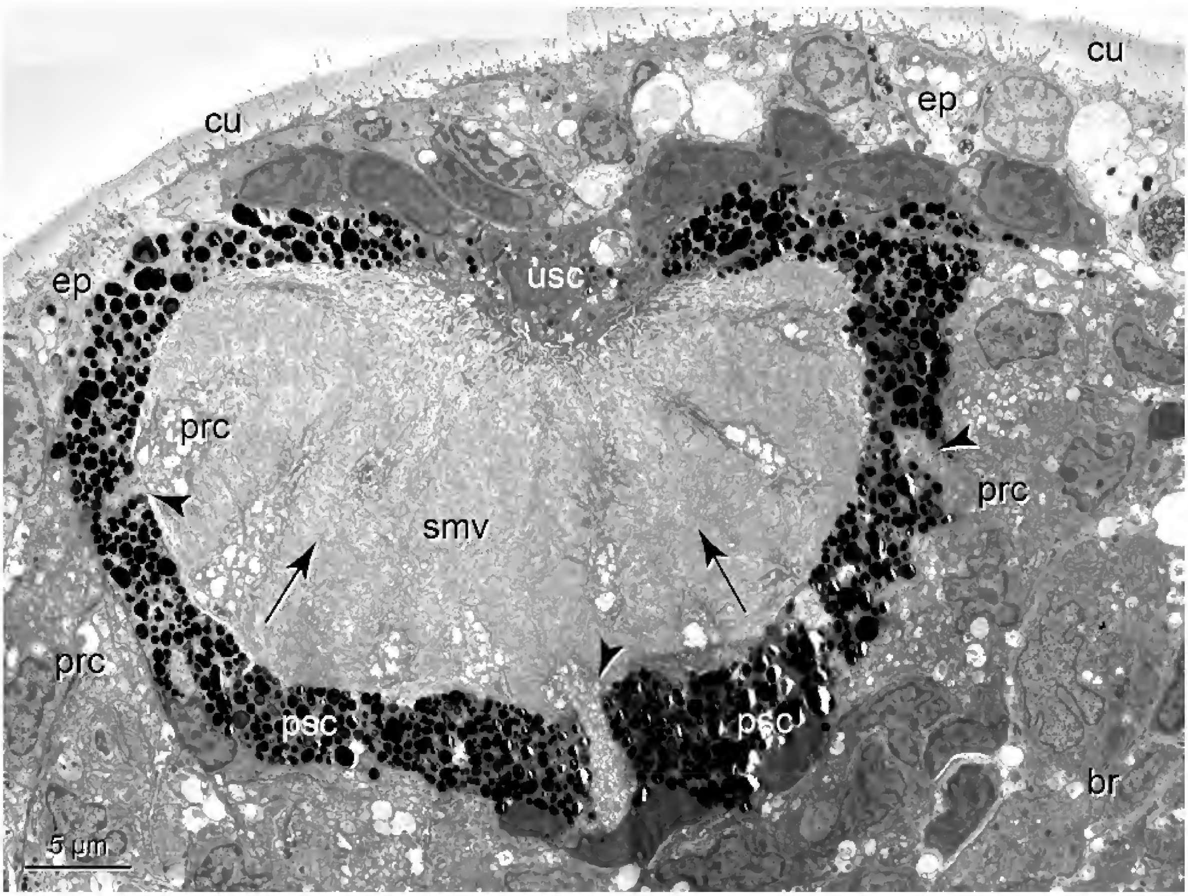

Figure 8. *Macrochaeta clavicornis* (Acrocirridae). 2nd pair of pigmented eye, typical multicellular adult eye with converse oriented photoreceptive processes (arrows), lens absent. Pigment cup formed by a layer pigmented supportive cells (psc) penetrated by processes of rhabdomeric photoreceptor cells (prc), pupil formed by unpigmented supportive cells (usc). Cu = cuticle, ep = epidermis, prc = photoreceptor cell, psc = pigmented supportive cell, smv = sensory microvilli, usc = unpigmented supportive cells. Original: I. Dykstra, Osnabrück.

comprising numerous neurites) which are situated on both circumoesophageal roots (fig. 11F). Some roots appear to be restricted to a smaller group of taxa such as roots Nos. 1, 2 and 3 which have only been found in Sabellariidae, Serpulidae and Sabellidae. On the other hand, roots No. 6 on the ventral and root No. 9 on the dorsal root of the circumoesophageal connective have been reported in most taxa investigated and may be promising candidates for having been present in the

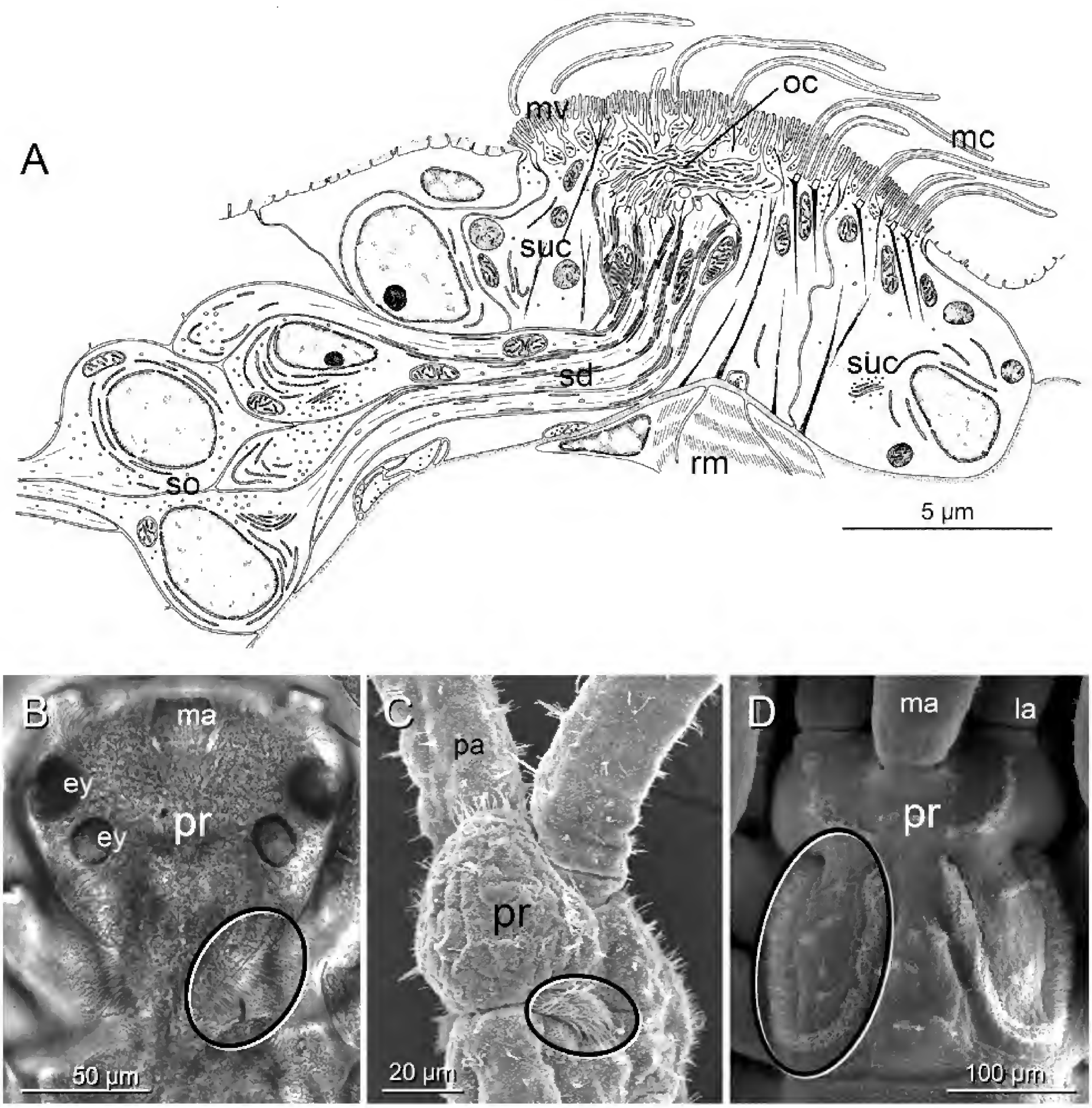

Figure 9. Nuchal organs. A. Schematic representation of nuchal organ in *Nerillidium troglochaetoides* (Nerillidae). After TEM observations, modified from Purschke (1997). B. *Eusyllis* (?) sp. (Syllidae). Nuchal organs (encircled) visible as ciliary patches in the posterior region of the prostomium, micrograph from living animal. C. *Saccocirrus* sp. (Saccocirridae). Nuchal organs form oval patches (encircled). D. *Myrianida prolifera* (Syllidae). Nuchal epaulettes form u-shaped ciliary band extending posteriorly on peristomium and 1st chaetiger. - ey = eye, la = lateral antenna, ma = median antenna, mc = motile cilium, mv = microvillus, oc = olfactory chamber, pa = palp, pr = prostomium, rm = retractor muscle, sd = sensory dendrite, so = soma of receptor cell, suc = supportive cell. C, D: SEM micrographs, W. Mangerich, S. Raabe, Osnabrück.

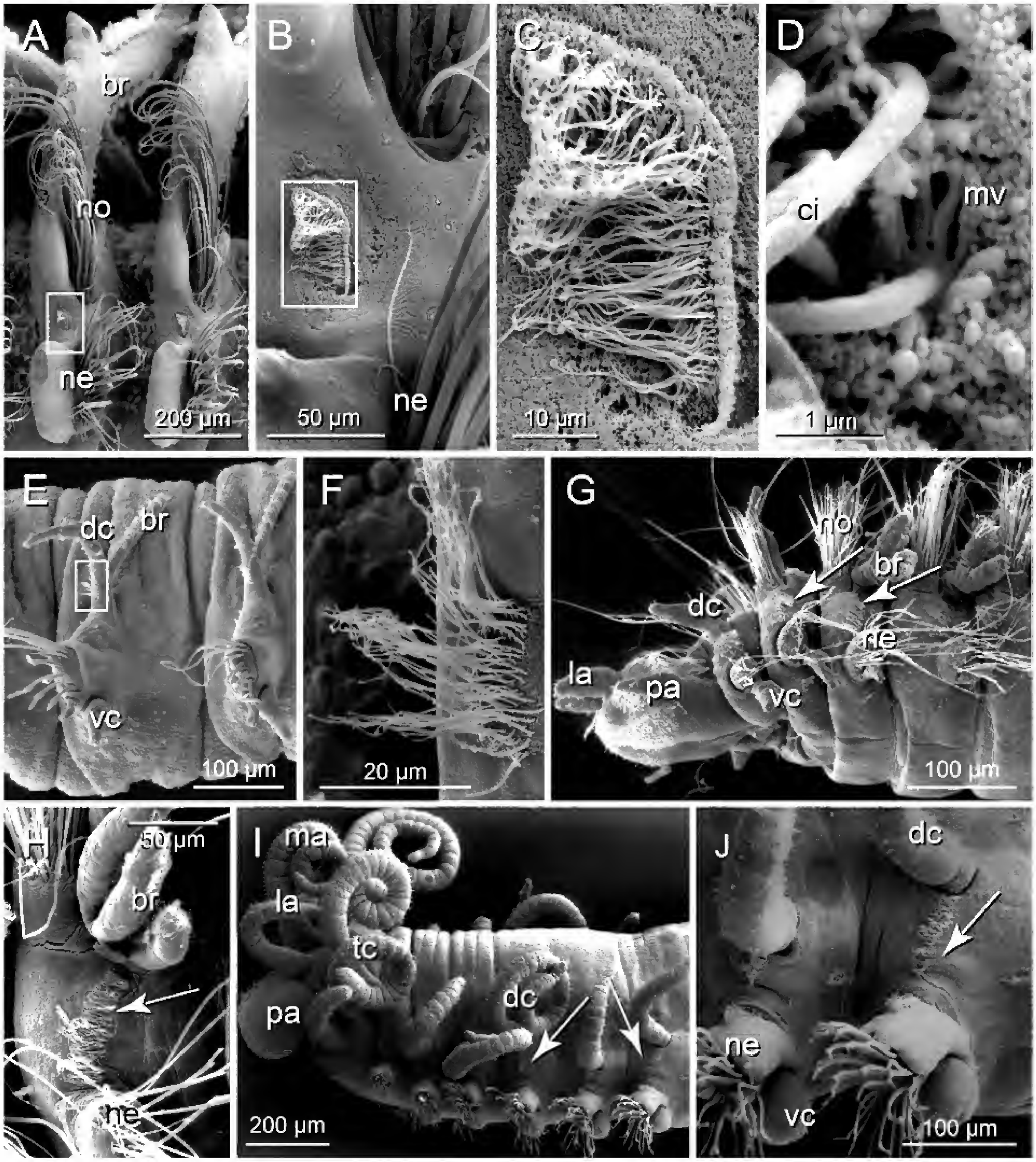

Figure 10. Lateral organs. A-D. *Malacoceros fuliginosus,* (Spionidae). A. 2 parapodia with lateral organ (boxed) between noto- (no) and neuropodium (ne). B. Enlargement of left parapodium, lateral organ visible as ciliary brush. C. Enlargement of B, note cilia arranged in distinct rows. D. Base of 2 collar receptors with single sensory cilium (ci) and circle of microvilli (mv). E-F. *Eunice pennata* (Eunicidae). Lateral organ (dorsal cirrus organ). E. 2 parapodia showing position of lateral organ (boxed). F. Enlargement of boxed area from E. G-J. Lateral organ like ciliary bands of unknown function between noto- and neuropodia in polychaetes. G-H. *Eurythoe complanata* (Amphinomidae). G. Anterior end, lateral view; arrows point to ciliary bands between noto- and neuropodia. H. enlargement of parapodium. I-J. *Syllis* sp. (Syllidae). I. Anterior end lateral view, parapodia with ciliary bands (arrows). J. Enlargement of parapodia with ciliary bands (arrow). - br = branchia, no = notopodium, ne = neuropodium, ci = cilium, mv = microvillus, dc = dorsal cirrus, vc = ventral cirrus, la = lateral antenna, pa = palp, ma = median antenna, tc = tentacular cirrus. SEM Micrographs; originals E, F M: Nesnidal, Osnabrück, G-J S; Raabe, Osnabrück

annelid ground pattern (Wilkens and Purschke, 2009b). Taxa regarded as belonging to the basal radiation (see Weigert et al., 2014) do not show a unique pattern but at least nerve root No. 6 is usually present. In developing and regenerating spionids the palps buds appear at the posterior edge of the prostomium and their peristomial position is achieved later on (e.g. Blake and Arnofsky, 1999; Lindsay et al., 2008). This feature may suggest a prostomial origin in general. Also in Apistobranchidae the palps are inserted in front of the nuchal organs and should therefore be prostomial. However, a prostomial origin may not be supported by observations in cirratulids (Petersen, 1999) where the palps originate more posteriorly.

Most of the palp-less taxa have been placed in Scolecida by Rouse and Fauchald (1997). They regard the absence of palps as the plesiomorphic character state in their "Scolecida-Palpata" hypothesis whereas in the "Errantia-Sedentaria" hypothesis absence is interpreted as a loss which must have happened more than once (Bartolomaeus et al., 2005, Struck, 2011; Struck et al., 2011; Weigert et al., 2014).

The entire prostomium is a highly sensory area innervated by a complicated network of nerves originating from the brain independent of the palp nerve roots (see Orrhage and Müller, 2005). Interestingly, such palp nerve roots have also been reported from taxa which do not possess palps (Scalibregmatidae, Paronidae and Orbiniidae; see Orrhage and Müller, 2005; Wilkens and Purschke, 2009b). This has been taken as an indication of reduction of palps in those taxa, rather than their primary absence. While representatives of several scolecidan taxa have not been investigated, preliminary investigations in Opheliidae (Purschke, unpubl. obs.) indicate occurrence of palp nerve roots in this family which contradicts previous studies (Orrhage, 1966). In certain species of Scalibregmatidae a secondary gain of palps from prostomial horns has been hypothesised based on a cladistics analysis (Martínez et al., 2013).

On the other hand, the tentacles present in the terebellomorph polychaetes Alvinellidae, Ampharetidae, Pectinariiidae, and Terebellidae have been regarded as representing multiple grooved palps (Rouse and Pleijel, 2001), even though from the structure of the anterior nervous system there is no evidence for the existence of palps and antennae in the latter three families (Orrhage, 2001). Instead it has been concluded that the tentacles of these belong to the alimentary canal and should be termed buccal tentacles (Orrhage, 2001). Moreover, their central nervous system appears to be highly derived and structurally simple (Orrhage, 2001; Heuer et al., 2010). However, this has been questioned by Zhadan and Tzetlin (2002). Likewise, a proof that the appendages in Siboglinidae are really palps is still lacking although normally assumed (see Rouse and Pleijel, 2001).

Eyes

Most annelids possess some kind of photoreceptor cells or light sensitive organ (Rouse and Pleijel, 2001; Purschke, 2005; Purschke et al., 2006). Due to their extreme structural diversity they have been regarded as difficult to evaluate in phylogenetic analyses (Fauchald and Rouse, 1997). There may be up to three different types of photoreceptor cells (PRCs): rhabdomeric PRCs, ciliary PRCs and phaosomous PRCs (Figs 7E-G, 8). The former two types, rPRCs and cPRC, occur with supportive cells either with shading pigment (PSC) or without pigment (USC). Only eyes (or ocelli) with PSC allow discrimination of the direction of light source (for reviews see Purschke, 2005; Purschke et al., 2006). These eyes may be divided into different types: larval and adult eyes (characterised by their molecular fingerprint and usually by their different structure) as well as cerebral and so-called ectopic eyes occurring elsewhere on the body (Purschke et al., 2006; Arendt et al., 2009). Depending on the taxa considered there may be 0, 1, 2 or 3 pairs of cerebral eyes (fig. 7A-C); and certain species may possess more eyes and sometimes in odd numbers (e. g. Terebellidae).

Larval type of eye. The so-called larval type of eye usually consists of only two cells: a PSC and an rPRC forming an inverse ocellus with the sensory processes projecting away from the incoming light (fig. 7F; Purschke, 2005; Purschke et al., 2006). In certain cases these eyes are still part of the epidermal epithelium and connected to the outside via a small pore (e. g. *Saccocirrus* spp. fig. 7F; see Arendt et al., 2009). Such ocelli are generally present in trochophores and may be formed and functional within 24 h after fertilisation (Dorresteijn, 2005). Such simple eyes are perfectly adapted sensory structures for positive or negative phototaxis (Jékely et al., 2008). Such eyes may occur in adults of certain species as well and, based on structural data, it is impossible to determine if they represent persisting larval eyes or diminutive adult eyes. With few exceptions of specialised eye types such larval type eyes have been regarded as being restricted to adults of sedentarian taxa (Purschke et al., 2006; Purschke and Nowak, 2013). The fate of the larval eyes in ontogeny is not completely known as it is, hard to follow especially in large species. Moreover, whereas formerly a replacement by the adult eyes has generally been assumed to occur besides rare cases of persistence (Purschke et al., 2006; Purschke and Nowak, 2013), recent investigations indicate probable persistence even in species for which a replacement by the adult eyes has been assumed (Backfisch et al., 2013). A unique example of larval eyes being transformed into adult eyes occurs in *Capitella teleta* (Yamaguchi and Seaver, 2013). So in this species the adult eyes are a mixture of both larval and adult eye structures and further studies are needed to determine how often this phenomenon occurs in other species.

Adult type of eye. Typical adult eyes in annelids are multicellular comprising rPRCs with shading pigment, PSCs and USCs. These cells form a continuous epithelium in which rPRCs and PSCs intermingle resulting in a converse (everse) eye with the sensory processes projecting towards the light (Figs 7D, F, G, 8; Purschke et al., 2006; Suschenko and Purschke, 2009). As these eyes develop from epidermal anlagen, they may still be connected with the exterior by a more or less prominent duct (Purschke and Nowak, 2013). Adult eyes of this kind are known to occur in Phyllodocida, Eunicida and Amphinomida, whereas lenses, which are typically formed by the PSCs, have only been found among Phyllodocida (Purschke et al., 2006; Suschenko and Purschke, 2009). Very likely, two pairs of adult eyes belong to the ground

pattern of Phyllodocida, Eunicida and Amphinomida. Given the phylogenetic hypothesis of Weigert et al. (2014) this means this is a plesiomorphic feature that has been lost secondarily in Sedentaria. On each side the eyes develop from a common anlage and split into two eyes each after initial formation (Dorresteijn, 2005; Backfisch et al., 2013). However, in these taxa several representatives exist which usually possess rather small eyes of unknown affiliation to either larval or adult eyes. This is especially the case for the so-called eyespots which are present in many representatives of Syllidae, but also occurs in several other members of these groups. So far only a few species have been investigated. Several examples of miniaturisation of adult eyes are reported in errant polychaetes (Purschke and Nowak 2013; Purschke unpubl. obs.).

In sedentarian polychaetes miniaturised adult eyes are present as well, for example in *Fauveliopsis* cf. *adriatica* and with respect to their proposed phylogenetic position more importantly in the orbiniid *Scoloplos armiger* (Wilkens and Purschke, 2009b; Purschke, 2011). The pigmented eyes of Sipunculida are also structurally similar to the adult eyes of polychaetes (Purschke, 2011), which are especially important in the "new annelid phylogeny" where Sipuncula are part of the annelid radiation (Dordel at al., 2010; Weigert et al., 2014). Among Sedentaria, Flabelligeridae and Accrocirridae are known to possess rather large eyes and should be examined to determine if they represent typical adult annelid eyes. Whereas Flabelligeridae have been described to possess an unusual platyhelminth type of pigmented eye of inverse design (see Purschke et al., 2006), preliminary observations in *Macrochaeta clavicornis* (Sars, 1835) (Accrocirridae), which possess three pairs of eyes, an anterior minute pair and two larger pairs situated more posteriorly, showed that the minute eye probably is a reduced adult eye. The second pair is an adult eye without doubt (fig. 8) and the most posterior pair is of the platyhelminth type. This implies that the inverse eye most likely represents a new acquisition in a taxon at least comprising these two families within Cirratuliformia. However, these studies have to be extended to more species of Cirratuliformia to test this hypothesis. Further investigations must show whether the small eyes present in other sedentarian annelids also represent miniaturised adult eyes. For *Capitella teleta* Blake et al., 2009 it may be that the eye is unique as it is a mixture of the larval and adult eye (Yamaguchi and Seaver, 2013). Also the findings in the leech *Helobdella robusta* (Shankland et al., 1991), fit into this general picture (Döring et al., 2013). It could be shown that the PRCs probably have been derived from those of the adult annelid eye, whereas the eyes as such evolved *de novo* in the stem lineage of leeches (e.g. fig. 7E).

In summary, gene expression studies support that the larval eye in annelids is homologous to the pigmented eyes of other bilaterians (e. g. under control of *pax6*; Arendt et al., 2002; Dorresteijn, 2005; Backfisch et al., 2013; Döring et al., 2013). At some point in the annelid lineage adult eyes must have evolved, no later than in the last common ancestor of Amphinomidae, Sipuncula and Pleistoannelida. Whether they might already belong to an earlier emerging lineage has yet to be determined and needs to be investigated in Oweniidae, Magelonidae, Apistobranchidae and Chaetopteridae which are regarded as belonging to the first, basal radiation in annelids (Struck, 2011; Weigert et al,. 2014). However, histological investigations of *Chaetopterus variopedatus* indicate that adult eyes are present (Martin and Anctil, 1984). Probably there are parallel events of miniaturisations and progressive reductions or losses of adult (and larval) eyes, one of which is characteristic for the lineage comprising most sedentary groups including Clitellata (Döring et al., 2013). Besides the pigmented eyes there are other photoreceptive structures, which may have a similar phylogenetic importance but further investigations are necessary (see Hausen, 2007; Wilkens and Purschke, 2009a).

Nuchal organs

Nuchal organs are situated at the posterior edge of the prostomium and are visible as densely ciliated structures, which can be withdrawn in many forms (fig. 9A-D) (Purschke, 1997, 2002, 2005). Especially in many burrowing, tube-building sessile or terrestrial forms they may be completely internalised. Despite their external diversity (fig. 9B-D) they show an overall structural similarity and are composed of a few identical cell types throughout (fig. 9A). Thus, their homology is generally accepted (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001; Purschke, 2005).

Whereas their absences in polychaetes usually were regarded as losses, the absence of nuchal organs in Clitellata was mostly seen as primary resulting in recognition of nuchal organs as the most important autapomorphy for the taxon Polychaeta (Rouse and Fauchald, 1997). On the other hand, there is evidence that there is a high probability that Clitellata have also lost nuchal organs (e. g. Purschke, 1997, 1999, 2000, 2002). Interestingly, all molecular phylogenetic studies conducted so far revealed Clitellata in a highly derived position among the polychaetes supporting the latter view (Weigert et al., 2014). By contrast, some taxa such as Oweniidae need to be re-examined to determine if nuchal organs are present as vestiges, or if they are really absent. Thorough investigations by Hausen (2001) confirmed the absence of nuchal organs in two species of *Magelona* and presence in Apistobranchidae. At present it remains unresolved whether these structures were present in the last common ancestor of Annelida or have evolved later within the annelids.

Lateral organs

Ciliated bands, papillae or pits which occur between noto- and neuropodia in many sedentary polychaetes represent sensory organs consisting of two types of uniciliated receptor cells and supportive cells (Purschke and Hausen, 2007). These organs are commonly termed lateral organs (fig. 10A-D). Besides sedentary polychaetes, such organs have been shown to be present in Eunicida as well, here called dorsal cirrus organ due to the lack of a typical notopodium in these taxa (fig. 10E, F; Hayashi and Yamane, 1997; Purschke, 2002). However, in Eunicida only one receptor cell type is present (Hayashi and Yamane, 1997; Purschke, unpubl. obs.). Similar ciliary bands have also been observed in representatives of Amphinomidae and Syllidae, but histological investigations are still needed (fig. 10G-J).

For a robust phylogenetic assessment of the evolution of lateral organs data of some important taxa is missing and especially their occurrence in representatives of the basal annelid radiation should be (re)investigated. According to the literature lateral organs are present in Magelonidae and Apistobranchidae but absent in Chaetopteridae and Oweniidae (Fauchald and Rouse, 1997). However, their fine structure is unknown. Given a questionable presence in amphinomids the resulting picture currently is puzzling allowing several equally parsimonious explanations, either as ground pattern character or as convergently evolved structures occurring in several lineages.

Central nervous system

The central nervous system in Annelida is generally described as a rope-ladder nervous system consisting of a prostomial brain connected with the ventral nerve cord via double circumoesophageal connectives (Bullock and Horridge, 1965; Orrhage and Müller, 2005; Müller, 2006; Lehmacher et al., 2014). The ventral nerve cord was generally seen as rope-ladder-like chain of paired segmental ganglia connected by connectives and commissures. However, as already stated by Bullock and Horridge (1965) a considerable degree of variation in polychaetes exists making it difficult to deduce phylogenetic hypotheses (fig. 11A-H).

Müller (2006) considered a nervous system with the following characters as the ground pattern in annelids: (1) paired circumoesophageal connectives consisting of dorsal and ventral roots interconnected via two intracerebral commissures each; (2) a ventral nerve cord comprising primarily five connectives; (3) numerous commissures per segment; (4) numerous segmental nerves per segment and (5) peripheral nervous system with several longitudinal pairs of nerves and one median unpaired nerve. The highest numbers reported so far are 17 longitudinal nerves in *Saccocirrus papillocercus* (see Orrhage and Müller, 2005) and up to 18 segmental nerves in *Polygordius appendiculatus* (see Lehmacher et al., 2014). Thus the entire nervous system has an orthogonal appearance and a typical rope-ladder-like nervous system is a rare exception or does not exist at all (fig. 11A-E). From this pattern all nervous system structures observed may have derived. For instance, the most common polychaete nervous system shows partly fused circumoesophageal connectives, whereas in clitellates they are completely fused forming simple connectives throughout (fig. 11C, D). Interestingly, during ontogenesis and regeneration experiments this fusion can be observed and each annelid nervous system starts with double circumoesophageal roots (e.g. Hessling and Westheide, 1999; Müller, 2004, 2006; Müller and Henning, 2004). The same applies for the structure of the ventral cord.

The question of whether the nervous system has a basiepithelial or subepidermal position in the ground pattern is still a matter for discussion. However, as already discussed (Bullock and Horridge, 1965; Martin and Anctil, 1984; Purschke, 2002; Orrhage and Müller, 2005) a basiepidermal position is more common than formerly thought. Interestingly, species with a subepidermal position of the nervous system in adults may have a basiepidermal position in juveniles (e.g., *Scoloplos armiger;* Purschke, unpubl. obs.). In this case ontogeny may reflect the direction of evolution for this character. In many species the ventral nerve cord usually lies between the ventral longitudinal muscle bands bulging into the body cavity but is still part of the epidermis as documented by a continuous ECM with the epidermis (Figs 5B, 11E). As a consequence circular muscle fibres are generally interrupted in this area (Lehmacher et al., 2014). Alternatively, the nerve cord may be subepithelial for a short distance allowing the circular fibres to pass below it.

Whether the nerve cord was actually a medullary cord (fig. 11B) and not subdivided into connectives and ganglia (fig. 11C, D) in the ground pattern is another point of discussion. However, this seems to be a comparatively rare case in Annelida occurring in highly derived annelids such as oligochaetous Clitellata and a few polychaetes such as *Polygordius* spp. (see Lehmacher et al., 2014). Although ganglia and connectives are reported to occur in *Chaetopterus variopedatus* (see Martin and Anctil, 1984), annelid species regarded to be part of the basal radiation should be reinvestigated for this character.

Within the polychaete brain several ganglia (neuropils encased by associated neuronal somata) may be distinguished (fig. 11F-G; Orrhage and Müller, 2005). There are more than 25 pairs in errant forms whereas especially in many sedentary species there are no distinguishable ganglia at all (Heuer et al., 2010). Thus, with a few exceptions the former authors discouraged any efforts of homologising ganglia in annelids. However, recently, the so-called mushroom bodies, which were first identified by Holmgren (1916) in polychaetes, came back into the phylogenetic discussion (fig. 11G, H; Heuer et al., 2010). Heuer et al. (2010) regarded mushroom bodies as an ancient structure already present in the annelid stem species and their absence in many annelids as reductions. Since typical mushroom bodies have only been shown to exist in Nereididae and Aphroditiformia and to a lesser degree in a few other errant taxa, as an alternative it has been proposed that mushroom bodies evolved within Errantia or even in one or some of their subtaxa as well as independently in arthropods (Struck, 2012; Struck et al., 2014). This view is held because so far these structures are unknown in any taxon regarded to be basal in the annelid radiation irrespective of which of the conflicting hypotheses is considered (fig. 2A, B).

Musculature

A body wall musculature consisting of an outer layer of circular and an inner layer of longitudinal fibres was generally considered to represent the annelid ground pattern (Dales, 1963; Pilato, 1981; Purschke and Müller, 2006). These muscles may be accompanied by other muscle systems such as oblique, diagonal, bracing and dorso-ventral fibres as well as muscles belonging to the parapodia. The existence of these different muscles indicates that the entire muscular system in annelids is highly diverse and complex. In the meantime, it is generally accepted that the longitudinal fibres do not form a complete cylinder rather they are arranged in discrete bands with four bands representing the ground pattern (fig. 5B; Rouse and Fauchald, 1995, 1997; Tzetlin and Filippova, 2005, Lehmacher et al., 2014). Usually the musculature is ventrally interrupted

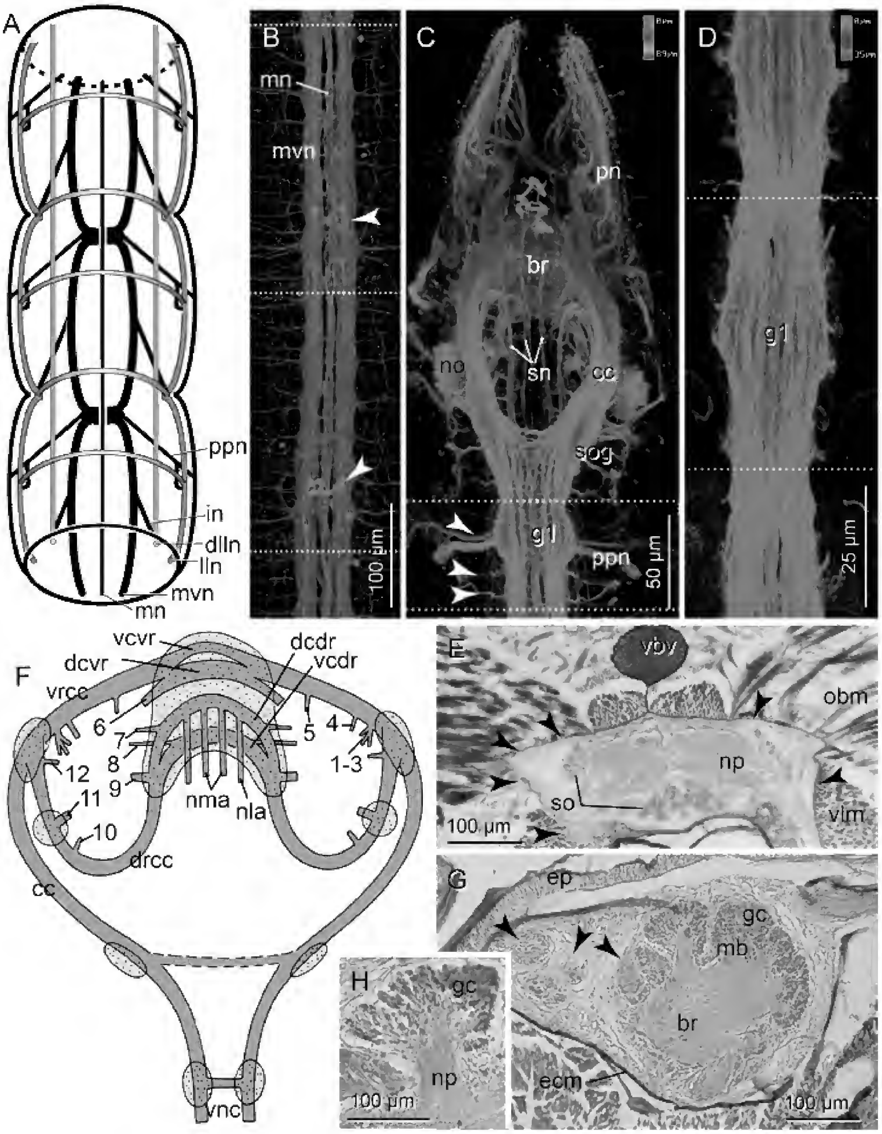

Figure 11. Nervous system and brain. A. Nervous system of the trunk with longitudinal and segmental circular nerves exemplified by *Parapodrilus psammophilus* (Dorvilleidae). Ventral cord consists of unpaired median (mn) and main paired nerves (mvn). B-D. Anti α-tubulin immunoreactivity; dotted lines indicate segment borders. B. *Polygordius appendiculatus* (Polygordiidae), ventral nerve cord (green) comprising three closely apposed neurite bundles, serotonergic perikarya (red) in a repetitive pattern although distinct ganglia are absent (medullary cord). Note high number of segmental nerves. C-D. *Brania clavata* (Syllidae); depth coding images. C. Brain (b) and ventral nerve cord in ventral view, ventral cord consists of several closely apposed nerves forming 3 bundles behind 1st ganglion (g1), 4 segmental nerves (arrowheads, ppn) in each segment; brain gives rise to several stomatogastric nerves (sn). D. Ventral cord in the trunk region. F. General diagram of the cephalic nervous system in polychaetes, numerals refer to palp nerve roots, somata stippled. E-H. *Nereis* sp. (Nereididae). E Ventral nerve cord in basiepithelial position (arrowheads refer to epidermal extracellular matrix). F. Parasagittal section with mushroom bodies (mb), note subepithelial position of brain; arrowheads point to cerebral ganglia. H Enlargement of anterior part of mushroom body with stalks of globuli cells (gc). – br = brain, cc = circumoesophageal connective, dcdr = dorsal commissure of drcc, dcvr = dorsal commissure of vrcc, dlln = dorsolateral longitudinal nerve, drcc = dorsal root of cc, ecm = extracellular matrix, ep = epidermis, g1 = 1st ganglion, gc = globuli cell, in = intestine, lln = lateral longitudinal nerve, mb = mushroom body, mn = median nerve of ventral cord, mvn = main nerve of ventral cord, nla = nerve of lateral antenna, nma = nerve of median antenna, no = nuchal organ, np = neuropil, obm = oblique muscle, pn = palp nerve, ppn = parapodial nerve, sn = stomatogastric nerve, so = somata of neurites, sog = suboesophageal ganglion, vbv = ventral blood vessel, vcdr = ventral commissure of drcc, vcvr = ventral commissure of vrcc, vlm = ventral longitudinal muscle, vrcc = ventral root of cc. A, F: modified from Müller and Orrhage (2005). Micrographs; B C: Lehmacher, C, D: M. Kuper, Osnabrück.

and separated by the ventral nerve cord and this may also apply to the circular fibres. These latter fibres are always less developed than the longitudinal ones and are likely to be absent in a number of taxa. Whether these absences are plesiomorphic or apomorphic is still being discussed and requires more data from a variety of polychaete taxa (see Tzetlin and Filippova, 2005; Purschke and Müller, 2006). Since these fibres are sometimes very delicate, investigations with modern methods such as cLSM are highly desirable (see Lehmacher et al., 2014). Recently, the oblique fibres running from the lateral sides to the ventral midline received closer attention and apparently their importance has been underestimated probably because the situation as present in earthworms had been regarded as representing the annelid ground pattern (see Purschke and Müller, 2006 for discussion).

Conclusions

In conclusion the question as to which characters belong to the last common ancestor of annelids has not been resolved although there has been considerable progress in recent years. Probably, the last common ancestor of annelids had a biphasic life cycle with a planktonic acoelomate larva and a benthic coelomate adult (including blood vascular system and metanephridia), a collagenous cuticle without being arranged in layers of parallel fibres, an epidermis with at least a few ciliated cells (responsible for generating water currents or movements of the animals), a homonomous segmentation, longitudinal muscle bands, ill-defined or lacking circular muscle fibres, oblique muscles running to the ventral midline, a nervous system comprising a prostomial brain and a ventral nerve cord comprising five connectives linked to the brain via double circumoesophageal connectives and additional longitudinal nerves that give the entire nervous system an orthogonal appearance, a foregut with dorsolateral ciliated folds (microphagous deposit feeder), a gut forming a straight tube, simple chaetae and parapodia and a head consisting of a prostomium and a peristomium with feeding palps, larval bicellular eyes and adult multicellular eyes.

Such adult eyes are not restricted to the errant forms and among the putative basal branching groups multicellular adult eyes are present at least in Chaetopteridae, Sipuncula and Amphinomida. A duplication event of the adult eyes possibly occurred in the stem lineage of Amphinomida and Pleistoannelida. There is a high degree of probability of parallel events of miniaturisations and progressive reductions or even losses of adult (and larval) eyes, one of which is characteristic for the lineage comprising most sedentary groups including Clitellata. The latter possess unique photoreceptor cells (phaosomes) derived from typical annelid rhabdomeric photoreceptor cells and occasionally secondarily developed pigmented eyes (fig. 7E; Döring et al., 2013).

Whether nuchal organs belong to the annelid ground pattern (Rouse and Fauchald, 1995, 1997) currently remains unresolved since their absence in Oweniidae, Chaetopteridae, Magelonidae and Sipuncula has yet to be confirmed. A similar scenario is conceivable for the lateral organs as well as for other characters such as the basiepithelial position of the ventral nerve cord and whether it is divided into ganglia and connectives or represents a medullary cord. In view of the new molecular phylogeny (Struck et al., 2011; Weigert et al., 2014) several members of the basal branching groups should be re-investigated to elucidate the characters of the annelid stem species.

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Molecular phylogenetics of the *Neanthes acuminata* (Annelida: Nereididae) species complex

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Abstract

Reish, D.J., Anderson, F.E., Horn, K.M. and Hardege, J. 2014. Molecular phylogenetics of the *Neanthes acuminata* (Annelida: Nereididae) species complex . *Memoirs of Museum Victoria* 71: 271–278.

The *Neanthes acuminata* (Nereididae) species complex is a broadly distributed group of marine benthic polychaetous annelids that is known by many names around the world and comprises at least four species. They are the only nereidids known that show exclusively male parental care. The female dies after laying her eggs in a common mucoid tube where they are fertilized, and the male incubates the eggs until the young leave the tube. All of the species in the *N. acuminata* complex are identical in their morphological characteristics and they all possess a similar number of segments and paragnath distribution and similarly shaped parapodia. However, populations from the U.S. East Coast, southern California, Hawaii and Portugal differ in chromosome number. Eye and egg colour also vary among populations—some worms in southern California have red eyes and produce bright yellow/orange eggs, while others have black eyes and produce pale yellow eggs. These variations suggest that *N. acuminata* may represent multiple evolutionarily significant units. Clarification of the phylogenetic relationships among lineages in this species complex will provide a framework for studying character evolution and revising taxonomy within this intriguing group of nereidids. To that end, we sequenced regions of one nuclear and two mitochondrial genes from worms sampled from multiple sites in North America (southern California, Mexico and Connecticut), the central Pacific (Hawaii) and Europe (Germany, Portugal and the UK). Maximum likelihood and Bayesian analyses of these data clarify relationships in this complex and show that worms sampled from California and Mexico represent two geographically intermingled subclades. These two subclades are congruent with eye and egg colour data; one subclade consists of red-eyed worms, the other consists of black-eyed worms. Furthermore, we found evidence that individuals representing these subclades can occasionally be found at the same locality.

Keywords *Neanthes caudata*, *Neanthes arenaceodentata*, polychaete, phylogenetic relationships, morphs, COI, 16S, ITS1.

Introduction

The polychaete *Neanthes acuminata* (Ehlers, 1868) (Annelida: Nereididae) species complex is cosmopolitan in distribution and comprises at least four species (Weinberg et al., 1990). *Neanthes acuminata* is the only valid scientific name for this group. It is known by this name from New England to North Carolina (Day, 1973). The southern California population was initially referred to the European species *N. caudata* (delle Chiaje, 1841) (Reish, 1957), which was later considered a synonym of *N. arenaceodentata* Moore, 1903 by Pettibone (1963). This name was applied to the California population by Reish and Alosi (1968), but Day (1973) considered both *N. caudata* and *N. arenaceodentata* synonyms of *N. acuminata*. *Neanthes cricognatha* (Ehlers, 1904), also considered part of the complex, is known from India and Hong Kong (Fauvel, 1950) and Australia and New Zealand. References to the literature concerning the populations used in this study are presented in Table 1. For convenience in this paper, *N. acuminata* refers to samples from New England; *N. caudata* to samples from Portugal, and *N. arenaceodentata* to samples from southern California, Mexico and Hawaii. All members of this species complex, except *N. cricognatha*, have been cultured through several generations in the laboratory at California State University, Long Beach (CSULB) by coauthor Reish (DJR). All are morphologically identical, with small conical paragnaths covering both rings of the proboscis and neuropodial heterogomph compound chaetae with a long blade terminating with a hook. Reproduction is unique in that the female reproduces once, but the male, which takes care of the embryos through the 21st segmented stage, is capable of reproducing as many as nine times (Reish et al., 2009). The

Table 1. Selected references to literature concerning the populations used in this study

Neanthes arenaceodentata:
Los Angeles Harbour Reish, 1956 [as *N. caudata*], Crippen and Reish, 1967, Reish, 1972
Venice, California: Winchell, et al. 2010.
Alamitos Bay: Reish, 1964 [as *N. caudata*], 1972
San Gabriel River, Reish, 1972, Oshida, et al. 1976.
Newport Bay: Reish, 1972
Punta Banda, Mexico: Díaz-Castañeda and Rodréguez-Villanueva, 1998
Hawaii: Bailey-Brock, et al., 2002.
Neanthes acuminata
Connecticut: Day, 1973, Weinberg, et. al., 1990
Neanthes caudata
Portugal: Fauvel, 1923, Bellan, 1967

members of the species complex differ from each other on the basis of chromosome number: *Neanthes acuminata* (2N = 22), *N. arenaceodentata* (2N = 18), Hawaiian *N. arenaceodentata* (2N = 28) (Weinberg et al., 1990*), N. caudata* (2N = 18) (Reish, unpublished) and behaviour (Sutton et al., 2005). Worms prefer to mate with worms collected from the same population. Males and females from southern California and New England, as expected, were aggressive to each other and failed to mate (Sutton et al., 2005). The chromosome number is unknown for *N. cricognatha*, which is not a part of the present study.

Materials and Methods

Taxon sampling

Neanthes acuminata was collected from the Connecticut intertidal zone by J. D. Hardege in 2004 and transported to CSULB where it was cultured for more than 10 generations before the culture was terminated. Collections from southern California were collected by DJR with exception of those from Venice Lagoon which had previously been cultured by Christopher J. Winchell at the University of California, Los Angeles. Collections from Estero Punta Banda and Bahia de San Quintin, Baja California were preserved in 70% ethanol by Maricarmen Necoechea and shipped to DJR. The Hawaiian specimens were collected by Bruno Pernet and shipped live to CSULB. Culturing these worms was unsuccessful and living specimens were preserved in 70% ethanol prior to death. Living specimens, except as noted above, were shipped by overnight express to the University of Hull and Southern Illinois University. Additional data on location, date of collection, culture history and locality are given in Table 2. Specimens from laboratory populations have been deposited in the Los Angeles County Museum of Natural History under the following catalog numbers: LACM-AHF 6194 (Reish lab), 6195 (L.A. Harbour), 6196 (Venice Canals), 6197 (Alamitos Bay), 6198 (San Gabriel River), 6199 (Newport Bay), 6200 (Hawaii) and 6201 (Faro, Portugal).

Culture methods were the same for all populations. Cultures were established by pairing a female, as determined by the presence of large eggs in her coelom, with a sexually unknown worm. A behavioral response was used to determine a male. Same sexes fight and opposite sexes lie alongside one another (Reish and Alosi, 1968). Males cannot be determined by the presence of sperm as in epitokal nereidids. Pairs were placed in a petri dish containing normal sea water and fed rehydrated dried *Enteromorpha* sp. and constructed a common mucoid tube. The female lays her eggs within the tube where they are fertilized. The female dies after egg laying or is eaten by the male. The male incubates the developing embryos by his body undulations, which refresh the water within the tube. After three to four weeks, the young worms (~21 segments) emerge from the parent's tube and commence feeding (Reish, 1957). There is no pelagic larval stage. Established populations were maintained in aerated 15-gallon (57 liters) aquaria containing 10 gallons (38 liters) of seawater. Approximately 100 juvenile worms were used to establish a population in an aquarium. Worms were fed weekly with commercial rabbit food that was soaked in seawater prior to use, stirred and the supernatant fluid added to the aquarium. Aquaria were drained and cleaned monthly. Worms reproduced within the aquaria and specimens were removed as needed.

DNA Extraction, PCR, and Sequencing

DNA was extracted from tissue samples using a DNeasy kit (Qiagen) according to manufacturer's instructions. Regions of two mitochondrial markers – cytochrome oxidase subunit I (COI or *cox1*) and the 16S ribosomal subunit (rrnL) – and one nuclear marker – internal transcribed spacer 1 (ITS1) – were amplified via PCR using HotStar Master Mix (Qiagen) (half reactions) using primer pairs (COI—Folmer et al., 1994; 16S—Geller et al., 1997; ITS1: ITS III and ITS VIII; Palumbi, 1996). PCR thermal cycling parameters were as follows: 95°C (15 minutes) for enzyme activation, followed by 35 cycles of 95°C (45 seconds), 40°C (45 seconds) and 68°C (1 minute) and a final terminal extension cycle of 68°C for 7 minutes. PCR products were purified using a MinElute Gel Purification Kit (Qiagen) and both strands were sequenced on an ABI 3730xl automated sequencer.

Molecular Data Set Construction

COI, 16S and ITS1 sequences were downloaded from the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore) for members of three other species; these were used as outgroups for our analyses. Two of these species were (like *Neanthes*) members of Nereididae—*Namalycastis jaya* Magesh, Kvist and Glasby (2012) (GenBank accession numbers: HQ456363 [COI] and HM138706 [16S]) and *Platynereis dumerilii* (Audouin and Milne-Edwards, 1834) (GenBank accession numbers [complete mitochondrial genome]: AF178678) A non-nereidid phyllodocid (*Nephtys sp.* 'San Juan Island' YV-2008, GenBank accession number [complete mitochondrial genome]: EU293739) was used as a distant outgroup to root the tree. These taxa were chosen because both COI and 16S data were available from the same

Table 2. Collection, culture and analysis location data on *Neanthes acuminata* complex

Collection locality	**Collection date**	**Eye/ova color**	**# lab generations**	**Lab**
Connecticut[1]	2002	Black/pale	~20	Hull
Venice, California	2008	Black/pale	~10	Hull, SIU
Los Angeles Harbour[2]	1964	Red/orange	200+	Hull, SIU
Los Angeles Harbour[1,3]	2008	Red/orange	~12	Hull. SIU
Alamitos Bay	2011	Black/pale	~4	Hull, SIU
San Gabriel River	2008	Black/pale	~12	Hull, SIU
Newport Bay	2004	Red/orange	~20	Hull, SIU
Bahia de San Quintin and Estero	2010	Unknown	Not cultured	Hull, SIU
Punta Banda, Baja California	2010	Unknown	Not cultured	
Oahu, Hawaii	2011	Black/pale	Not cultured	SIU
Portugal	2009	Black/pale	~8	Hull, SIU
Humber Estuary, UK[4]				Hull
Bremerhaven Estuary, Germany[4]				Hull

[1]No longer in culture
[2]Collected from the inner harbour (Reish lab)
[3]Collected from the outer harbour
[4]*Nereis diversicolor*

voucher specimen for all three species (ITS1 data from the outgroups were not included in our analyses; ITS1 sequences from species outside the *Neanthes acuminata* complex could not be aligned with ingroup ITS1 sequences).

Sequence Alignment and Phylogenetic Analyses

Sequence contigs were assembled and edited using Sequencher 5.1 (GeneCodes, Ann Arbor, Michigan), aligned with MUSCLE v3.8.31 (Edgar, 2004) and concatenated in Mesquite (Maddison and Maddison, 2010). ITS1 sequences from the outgroups (*Namalycastis* and *Platynereis*) were highly divergent from *Neanthes acuminata* ITS1 sequences, leading to spurious preliminary alignments. As a result, we excluded outgroup ITS1 data from the data matrices prior to alignment. Preliminary analyses suggested that the individual loci supported topologically concordant phylogenies, so data from the three individual loci were concatenated into two data matrices—a "full" data set (comprising all specimens for which at least one locus—COI, 16S or ITS1—was sequenced), and an "all three genes" data set (comprising all specimens for which COI, 16S and ITS1 sequences were generated). Four data partitioning schemes were evaluated for these data sets: 1) no partitioning (i.e., one data subset), 2) partitioned by gene (three data subsets), 3) partitioned by gene, with first and second codon positions of COI separated from third codon positions (four data subsets: COI positions 1 and 2, COI position 3, 16S and ITS1) and 4) partitioned by gene with COI partitioned by codon (five data subsets; ITS1, 16S, COI 1st, 2nd and 3rd codon positions). Best-fitting substitution models were chosen using jModelTest v2.1.1 (Darriba et al., 2012) and the best-fitting partitioning scheme was chosen using a second-order correction of the Akaike information criterion (AICc) and the Bayesian Information Criterion (BIC). Partitioned maximum likelihood analyses were performed with GARLI 2.0 (Zwickl, 2006). The ML tree search consisted of 10 searches (5 with random starting trees and 5 with stepwise starting trees, each with 100 search replicates); ML bootstrap analysis in GARLI 2.0 comprised 100 pseudoreplicates, each with random starting trees and 10 search replicates. Bayesian analyses were performed with MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003), with four independent runs of four chains each, temperature set to 0.05 to improve mixing, and the run automatically terminated when a topological convergence diagnostic (the average standard deviation of split frequencies) dropped below 0.01. For Bayesian analyses, data were unpartitioned or partitioned by gene and codon position, as described for ML analyses.

Results

DNA was extracted from a total of 115 specimens. Due to difficulty in PCR amplification of some loci from some specimens and missing sequences for some loci for some outgroup taxa, the full data set comprises a substantial amount of missing data (Table 2). GenBank numbers for the sequences generated in this study are COI: KJ539071 - KJ539141, 16S: KJ538962 - KJ538996, ITS1: KJ538997 - KJ539070. The full and "all three genes" data matrices are available on request to FEA.

The best-fitting models for each of the partitions were as follows—COI 1st positions: 000010+G, COI 2nd positions:

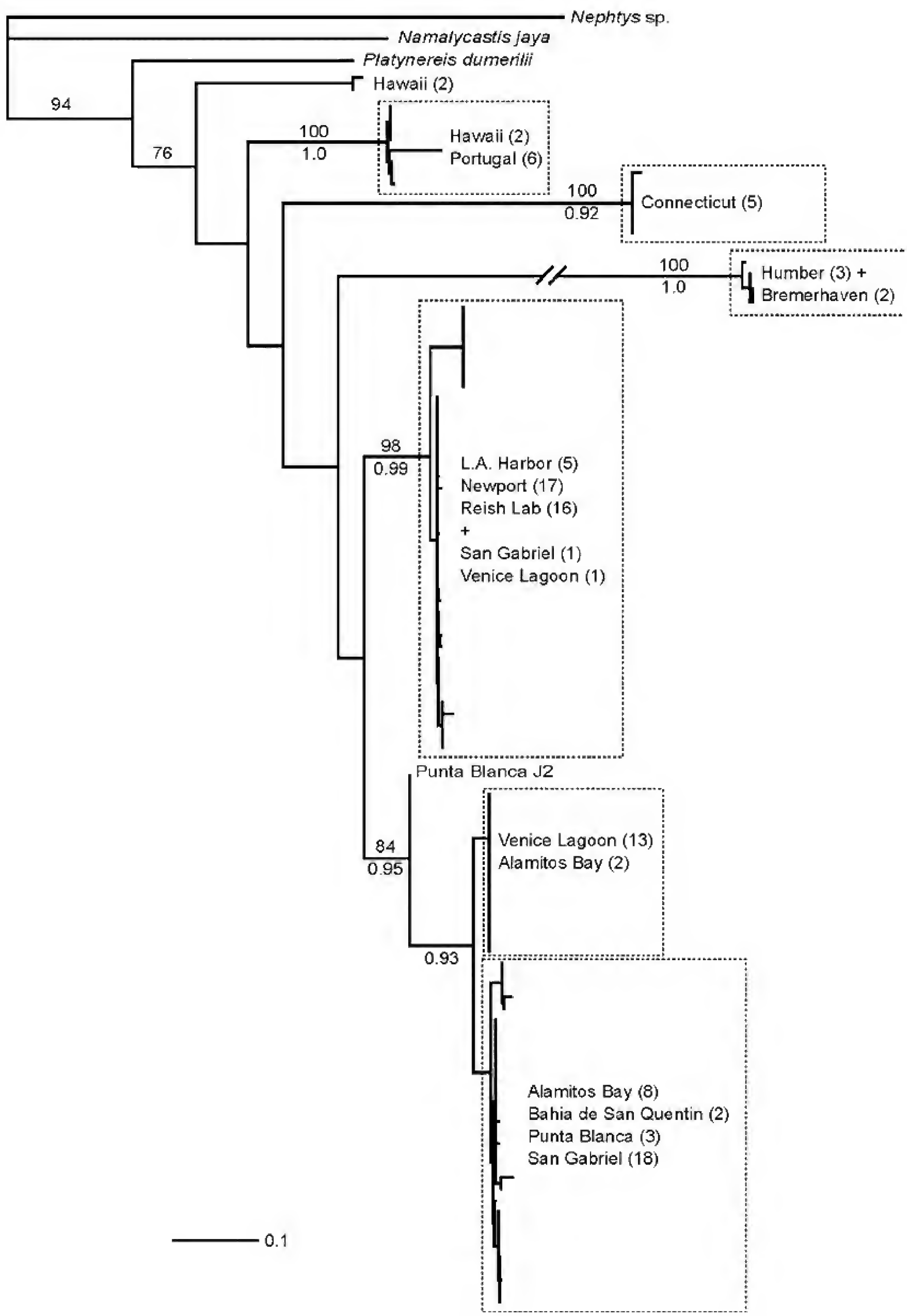

Figure 1. Maximum-likelihood tree resulting from partitioned analysis of the full concatenated data set in Garli 2.0. Numbers above branches represent ML bootstrap support values; numbers below branches represent posterior probabilities from an unpartitioned Bayesian analysis in MrBayes 3.2.1. Within each dashed box names refer to all populations present within that clade.

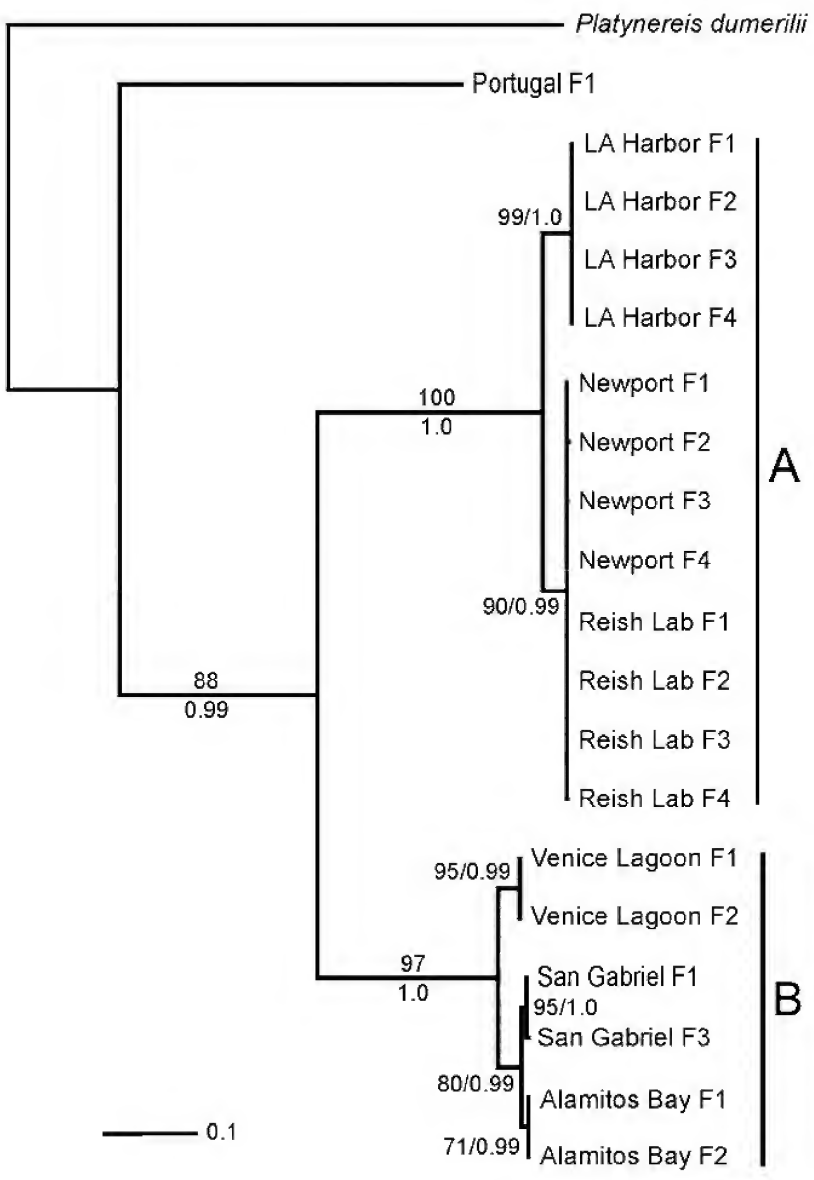

Figure 2. Maximum-likelihood tree resulting from partitioned analysis of the "all three genes" data set in Garli 2.0. Numbers above branches represent ML bootstrap support values; numbers below branches represent posterior probabilities from an unpartitioned Bayesian analysis in MrBayes 3.2.1

Table 3. Number of specimens (OTUs) and amount of missing data for the "full" and "all three genes" data matrices.

	Matrix	
	Full	**All three genes**
Number of OTUs	111	20
# outgroup OTUs	3[a]	1[b]
OTUs missing		
COI	37	0
16S	73	0
ITS	37	1[c]
OTUs with only:		
COI	21	0
16S	6	0
ITS	28	0
Total missing datad	56%	7.6%

a - *Nephtys* sp., *Namalycastis jaya* and *Platynereis dumerilii*
b - *Platynereis dumerilii* only
c - Missing from *Platynereis dumerilii*
d - includes terminal gaps, missing loci and indels in ITS

000001 (full) and 011110+F (all three genes), COI 3rd positions: HKY+G, 16S: TPM2uf+G (full) and 011212+G+F (all three genes), ITS1: K80+I (full) and 011010+G (all three genes) (substitution codes are from jModeltest; the TPM2uf model is the "three-parameter" or Kimura (1981) model, and has a substitution code of 010212). The best-fitting partitioning scheme for both the full and all-three-genes data set was the "five data subsets" scheme in which 16S, ITS1 and each COI codon position had a separate substitution model. Phylogenies resulting from maximum likelihood and Bayesian analyses of the "full" and "all three genes" data matrices under this partitioning scheme are presented in figures 1 and 2. Partitioned Bayesian analyses of the full data set failed to converge after >20 million generations; posterior probabilities shown in figure 1 are from an unpartitioned Bayesian analysis (GTR+I+G model) of that data set. The overall topologies of the trees are consistent with one another, with several well-supported nodes in both trees.

Sequences could not be obtained for all loci from every worm; in the full data matrix, 56% of the cells were missing data (including alignment gaps). In some cases, individual worms were represented by data from only one or two loci, resulting in nonoverlapping data among specimens. For example, for the four Hawaii specimens, we obtained only 16S data for two specimens, only ITS1 data from another specimen and both COI and ITS1 data (but not 16S) from a fourth specimen. The closest match to the two Hawaii 16S sequences was a 16S sequence from a Portugal specimen, resulting in a closer (but artifactual) relationship between these two Hawaii specimens and the other two in the data set (Fig. 1). Despite this, trees resulting from maximum likelihood and Bayesian analyses of individual-gene data sets (not shown) and the "full" and "all three genes" concatenated data sets were congruent, so we will focus on trees resulting from analyses of the full data matrix. The full matrix trees suggested that worms sampled from Connecticut, Hawaii and Portugal, as well as *N. diversicolor* Müller sampled from Germany and the UK, were genetically distinct from one another, and all analyses recovered a well-supported clade comprising all worms collected from Mexico and California (Fig. 1). This clade comprised two well-supported subclades, one consisting of worms collected from Los Angeles Harbour and Newport Beach (clade A), and the other consisting of worms sampled from all other southern California and Mexico (Punta Banda) population (clade B). Two members of Clade A (San Gabriel JDH 12 and Venice Lagoon JDH 1) were collected in localities generally inhabited by Clade B individuals.

Discussion

The finding that worms sampled from Connecticut, Hawaii, southern California, and Portugal form separate clades on our trees that correlate with geographic location is not particularly surprising. Some studies of polychaete species complexes with broad geographical distributions have yielded genetic evidence of substantial cryptic variation (e.g. *Neanthes diversicolor*; Virgilio et al., 2009), while others have revealed little genetic differentiation among widely separated regions (e.g., Ahrens et al., 2013). Unlike many polychaetes (but similar to *N. diversicolor*), species in the *Neanthes acuminata* complex have no pelagic larval stage. Species in this complex also use odour to initiate interpopulation aggression and pre-mating isolation. These life history features could partially explain why we seem to see genetic differences over short geographic distances. Worms sampled from Connecticut (2n=22) and Hawaii (2n=28) have different diploid chromosome numbers than do worms from California (2n=18), corroborating the inference that at least these three clades of worms in our phylogeny (Fig. 1) represent distinct species.

Two distinct subclades were found in southern California, one consisting of specimens collected from Los Angeles Harbour and Newport Beach (clade A), and the other comprising samples from all other southern California and Mexico sites (clade B). These clades were congruent with morphological and karyotypic differences seen among these populations—worms in clade A have red eyes and bright yellow/orange eggs, while worms in clade B have black eyes and pale yellow eggs. Since there are only two known populations in the *N. acuminata* complex with this colour distinction, we propose that the specimens from Los Angeles Harbour and Newport Bay were the result of a mutation giving rise to red eyes and bright orange ova. A similar eye colour mutation arose in a laboratory population of *Platynereis dumerilii* that was maintained for a long period of time at the Universität Köln (Fischer, 1969). The orange eye color was the result of a mutation that generated a recessive *or* allele. Backcrosses between black-eyed worms and the mutant form with red eyes produced a 1:1 ratio of black-eyed/orange-eyed

offspring. We speculate that a mutation producing the red eye/bright orange ova occurred in Newport Bay population which occurs intertidally in the back bay area. This mutant population may have been accidentally introduced into Los Angeles Harbour. It was a common practice for the owners of pleasure boats docked in Newport Bay to move their boats into the polluted waters of the inner harbour of the Los Angeles area to kill the fouling organisms attached to the vessel. Since *N. arenaceodentata* is known to live within the fouling organism community attached to boat floats (Crippen and Reish, 1969), the mutant could have been associated with such organisms attached to pleasure boats anchored in Newport Bay and were transported to Los Angeles Harbour in this way. The initial collection of *N. areaceodentata* was made in the west basin area of Los Angeles Harbour in December 1953 by DJR. This collection formed the basis of the life history study of the species (Reish, 1957). The ova were bright orange but the eye color was not noted. This population was destroyed prior to DJR moving to CSULB.

Additional evidence for the Newport-Los Angeles Harbour clade is the behavioural responses observed in the Southern California populations by Sutton et al. (2005). Black-eyed San Gabriel River worms showed more aggression toward red-eyed worms sampled from two sites (Newport and LA Harbour) than worms from the two red-eyed populations showed toward each other (though these findings were not statistically significant). Earlier Weinberg et al. (1992) reported that the inability of worms from the lab population to mate with worms from San Gabriel River and Newport Bay was evidence for rapid reproductive isolation of the lab worms following a founder event. However, this hypothesis was rejected by Rodriguez-Trelles et al. (1996) based on allozyme electrophoresis analyses of the three populations. Worms from the lab population and San Gabriel River produced offspring (DJR, personal observations). Worms collected from another locality in LA Harbour in 2008 by DJR were identical in appearance to the lab population, indicating little or no change from the 1964 collection.

The second clade on the Pacific Coast comprises worms from Venice, Alamitos Bay, San Gabriel River and Baja California. There are many estuaries in Southern California and Baja California and many of them have been altered in California, but those in Baja California have not been modified to any great extent. We assume that populations have existed in these areas for a long period of time. Historically, Alamitos Bay was an estuary formed by the San Gabriel River, but it became separated following a flood in 1938. The San Gabriel River became polluted and was devoid of benthic life by the late 1950s (Reish, 1956). Subsequently, the sources of pollution were eliminated and the channels were deepened. Shortly thereafter, electricity-generating plants were constructed and water was taken from Alamitos Bay for cooling the plants and discharged into San Gabriel River. *Neanthes arenaceodentata* was not found in San Gabriel River until 1971 (Reish unpublished report); we assume that they were introduced from Alamitos Bay.

Two members of Clade A (San Gabriel JDH 12 and Venice Lagoon JDH 1) were collected in localities generally inhabited by Clade B individuals, suggesting that limited migration occurred among these sites.

In conclusion, we have demonstrated that members of the *N. acuminata* complex sampled from multiple sites in the U.S., Mexico and Europe represent genetically distinct groups (possibly distinct species), and the different morphs of *N. arenaceodentata* seen in southern California represent two genetically distinct groups. We believe that the different populations seen in southern California may be the result of limited larval dispersion, the use of signature odour profiles for interpopulation aggression, pre-mating isolation, and preference for an estuarine habitat.

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Fishing bait worm supplies in Japan in relation to their physiological traits

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Abstract

Saito, H., Kawai, K., Umino, T. and Imabayashi, H. 2014. Fishing bait worm supplies in Japan in relation to their physiological traits. *Memoirs of Museum Victoria* 71: 279–287.

Market research was conducted from 2009 to 2013 to investigate the supply of live worms for fishing bait in Japan. We obtained 25 types of live fishing bait worms, including 16 species of polychaete, 1 species of echiuran, and 1 species of sipunculid. These were divided into three groups according to their country of origin: 1) worms supplied from native populations, five species (*Perinereis wilsoni*, *Hediste diadroma*, *Kinbergonuphis enoshimaensis*, *Pseudopotamilla occelata*, and *Hydroides ezoensis*), 2) worms supplied from both native and non-native populations, three species (*Marphysa* cf. *iwamushi*, *Halla okudai*, and *Urechis unicinctus*), and 3) worms supplied from non-native populations, 10 species (*Perinereis linea*, *Alitta virens*, *Nectoneanthes uchiwa*, *Namalycastis rhodochorde*, *Glycera nicobarica*, *Diopatra sugokai*, *Marphysa* cf. *tamurai*, *Marphysa* cf. *mossambica*, *Scoletoma heteropoda*, and *Sipunculus nudus*). Salinities in which no mortality of nereid worms occurred was 5–35 psu in *Alitta virens*, 5–30 psu in *Namalycastis rhodochorde*, and 10–35 psu in *Perinereis linea*. Worms living in temperate areas had a wide temperature tolerance of 5–30 °C in *Alitta virens*, *Perinereis linea*, *Glycera nicobarica*, *Marphysa* cf. *iwamushi*, and *Scoletoma heteropoda*. Tropical species (*Namalycastis rhodochorde* and *Marphysa* cf. *mossambica*) could not survive above 20 °C.

Keywords endangered species, fishing bait, import, non-native species, polychaete

Introduction

Human-mediated introduction of aquatic organisms beyond their native range has long been of great interest to ecologists. Although the shipping industry has received considerable attention as a dispersal mechanism for aquatic nuisance species, many invasions have been linked to other mechanisms of transfer including the bait industry (Weigle *et al*., 2005). The release of unused bait by anglers is an important vector of invasive species (Haska *et al*., 2012; Kilian *et al*., 2012). Previous studies reported that live fishing bait has been imported from Asian to European countries and the USA (Olive, 1994; Gambi *et al*., 1994; Costa *et al*., 2006; Cohen, 2012). Since 1969, about 1,000 t a year of live fishing bait has been imported into Japan from mainly Asian countries (Hayashi, 2001). According to a review of human-mediated introduction of aquatic organisms into Japan (Iwasaki, 2006), one *Perinereis* and one *Marphysa* species have been imported as bait. However, a preliminary investigation revealed that there are unconfirmed bait species other than these two worms in the Japanese bait market, and therefore detailed research is needed to clarify how many species are supplied as live fishing bait.

In this study, market research was conducted from 2009 to 2013 to investigate the supply of live bait worms in Japan. In addition, to determine which species pose the greatest risk as invasives, we studied some of the physiological traits (salinity and temperature tolerances) of the imported bait species.

Materials and methods

Market research

Bait worms were purchased at stores and wholesalers in Hiroshima, Okayama, Osaka, Toyohashi, and Hamamatsu, or via online from Osaka, Nagoya, and Sendai from December 2009 to May 2013. At the same time information on the local fishing name, source country, price, commercial size, and target fishes was obtained (Fig. 1). Bait worms were fixed in 10% formalin and then stored in 70% ethanol. Specimens were identified under stereo and compound light microscopes. All specimens were deposited at the Laboratory of Aquatic Animal Ecology, the Graduate School of Biosphere Science, Hiroshima University.

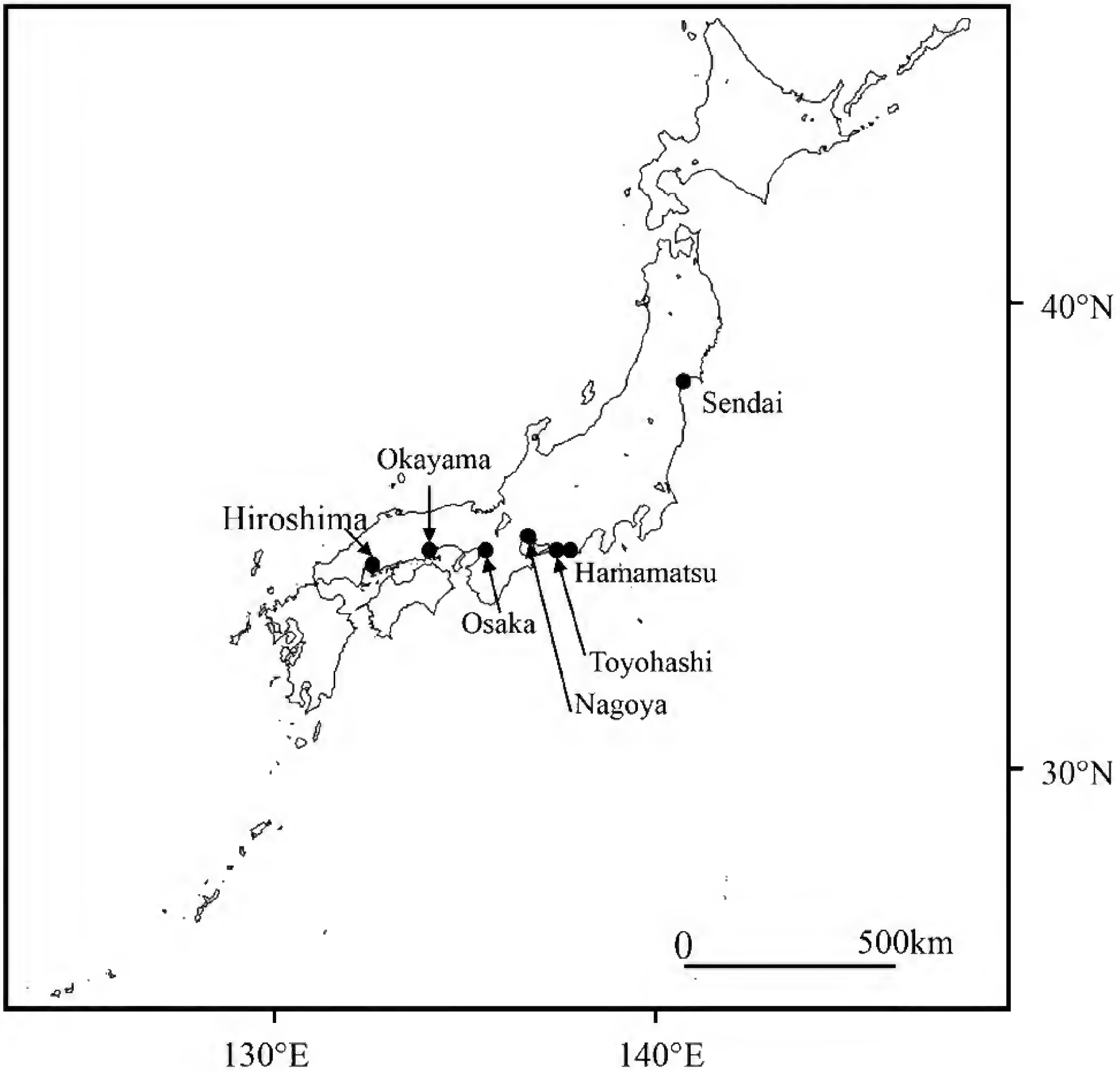

Fig. 1. Location of market research in Japan.

Survival experiments

Eight species of imported worms, *Perinereis linea, Alitta virens, Namalycastis rhodochorde, Glycera nicobarica, Marphysa* cf. *iwamushi, Marphysa* cf. *mossambica, Scoletoma heteropoda*, and *Halla okudai* were obtained from bait shops. Each species of worm was transferred to an aerated 120-L aquarium containing artificial seawater at 25 ppt and 23 °C for 24 h before experiments to exclude inactive individuals.

Survival experiments were performed in polyethylene containers (30×12×9 cm) maintained in a biotron (UZ-2242, NK system). Each experimental worm was placed in a container provided with a 5-cm layer of artificial seawater with an air filter. Experiments were replicated five times at seven different salinities (5, 10, 15, 20, 25, 30, and 35 ppt) at 23 °C, and seven different temperatures (5, 10, 15, 20, 25, 30, and 35 °C) at 25 ppt. The inhabitable range of salinity and temperature was evaluated from the 100 % survival of individuals after 5 days.

Table 1. Local market names, research dates, market places, source country, commercial size, price, and target fish of the bait worms sold in Japan.

Local market name	Research date	Market place	Source country	Commercial size (BL: cm)	Price (Japanese Yen)	Target fish*
Ishi-gokai	14 May 2010	Hiroshima	Japan	5-10	800 /100g	Whiting, Goby
Ao-mushi	17 Mar. 2010	Hiroshima	China	10-20	600 /100g	Flounders, Greenling
Super Ao-mushi	31 Aug. 2011	Osaka via online	China	10-20	750 /100g	Flounders, Greenling
Aka-isome	31 Aug. 2011	Osaka via online	China	10-20	750 /100g	Flounders, Greenling
Mizu-gokai	11 Mar. 2012	Okayama	Japan	10-20	1000/100g	Goby, Japanese Seaperch
Ao-kogane	16 Dec. 2009	Hiroshima	Netherlands	15-25	800 /100g	Flounders, Black Seabream
Aka-kogane	17 Nov. 2010	Osaka	China	15-25	1500 /100g	Flounders
Super Cordelle	23 Jul. 2012	Sendai via online	Vietnam	30-80	300 /indv.	Red Seabream
Chi-mushi	10 Jan. 2010	Osaka via online	China	15-25	750 /100g	Flounders
Shiro-chirori	21 Mar. 2013	Hamamatsu	China	15-25	1000 /100g	Flounders
Fukuro-mushi	11 Aug. 2010	Osaka	China	10-20	1600 /100g	Black Seabream
Iso-mushi	26 Mar. 2011	Toyohashi	Japan	10-20	50 /indv.	Black Seabream, Whiting
Iwa-mushi	9 May 2013	Hiroshima	Japan	15-25	2000 /100g	Flounders, Greenling
Hon-mushi	18 Feb. 2013	Hiroshima	China	15-25	2000 /100g	Flounders, Greenling
Honsa-mushi	10 Jan. 2010	Osaka via online	China	20-40	2000 /100g	Flounders, Greenling
Straw-mushi	23 Sep. 2012	Nagoya via online	Indonesia	15-25	100 /indv.	Black Seabream
Chrori	2 Jun. 2010	Hiroshima	China	15-25	1600 /100g	Whiting, Red Seabream
Tai-mushi	21 Dec. 2009	Hiroshima	China	30-60	6500 /100g	Red Seabream
Tai-mushi	8 May 2012	Hiroshima	Japan	30-60	6500 /100g	Red Seabream
Erako	16 Jan. 2011	Sendai via online	Japan	4-8	100 /100g	Flounders
Pipe-mushi	15 Nov. 2012	Osaka	Japan	1-2	100 /100g	Black Seabream
Kouji	16 Dec.2009	Hiroshima	Japan	5-10	190 /indv.	Red Seabream, Flounders
Super Kouji	12 Dec.2009	Hiroshima	China	10-15	110 /indv.	Red Seabream, Flounders
Yu-mushi	16 Dec.2009	Hiroshima	China	10-15	110 /indv.	Red Seabream, Flounders
BB Worm	10 Jan. 2010	Osaka via online	China	10-20	80 /indv.	Flounders

* Whiting: *Sillago japonica*, Goby: *Acanthogobius flavimanus*, Flounders: *Pleuronectes yokohamae* and *Kareius bicoloratus*, Greenling: *Hexagrammos otakii*, Japanese Seaperch: *Lateolabrax japonicus*, Black Seabream: *Acanthopagrus schlegelii*, Red Seabream: *Pagrus major*

Results

Market research

Twenty-five types of worm were sold as live fishing bait (Table 1). Of these, 17 were imported from China, Vietnam, Indonesia, and the Netherlands, and the remaining eight were supplied from Japan. Of the bait worms sold, 16 species were polychaetes, 1 species an echiuran, and 1 species a sipunculid. These were divided into three groups according to their country of origin. The bait characteristics of each species are described overleaf. (Table 2)

1) Worms supplied from native populations

Perinereis wilsoni Glasby and Hsieh, 2006

This species is a nereid worm, which was known as *Perinereis nuntia vallata* Grube (1857) (e.g., Imajima, 1996), but then described as a new species by Glasby and Hsieh (2006). *Perinereis wilsoni* is distributed on intertidal reef flats or rocky shores under boulders in Taiwan, China, Japan, and South Korea (Glasby and Hsieh, 2006). It has been cultured since the 1980s in Japan (Yoshida, 1984), and the worms have been mainly supplied under the local market name 'Ishigokai', meaning boulder worm. Wholesalers reported that a small amount of 'Ishigokai' is imported from China; however, we were unable to obtain Chinese specimens.

Table 2. Scientific names, local market names, distributions and habitats of bait worms sold in Japan.

Scientific name	Local market name	Distribution	Habitat
Perinereis wilsoni	Ishi-gokai	Taiwan, China, Japan, South Korea	Reef flats of Intertidal zone
Perinereis linea	Ao-mushi, Super Ao-mushi, Aka-isome	China, Korea	Mudflats of the upper intertidal zone in estuaries
Hediste diadroma	Mizu-gokai	Japan, China	Mud and Sandflats of intertidal zone in estuaries
Alitta virens	Ao-kogane	Northern Atlantic and Pacific oceans, North Sea	Mud and Sandflats of intertidal to subtidal zone in estuaries and coasts
Nectoneanthes uchiwa	Aka-kogane	Western Japan Korea, China	Mudflats in intertidal or subtidal zone in estuaries and coasts
Namalycastis rhodochorde	Super Cordelle	Vietnam, Indonesia, Malaysia	Mudflats of intertidal zone in estuaries with mangrove
Glycera nicobarica	Chi-mushi, Shiro-chirori	Southern Pacific and Indian Oceans, Japan, East China Sea	Sandflats of intertidal to subtidal zone
Diopatra sugokai	Fukuro-mushi	Malaysia, Thailand, China, Taiwan, Japan	Sandflats of intertidal to subtidal zone in estuaries and coasts
Kinbergonuphis enoshimaensis	Iso-mushi	Japan	Sand beach of intertidal zone of open sea
Marphysa cf. *iwamushi*	Iwa-mushi, Hon-mushi	China, Korea, Japan	Sandflats and rocky shores of intertidal to subtidal zone
Marphysa cf. *tamurai*	Honsa-mushi	East China Sea, Japan	Mud and Sandflats of intertidal zone
Marphysa cf. *mossambica*	Straw-mushi	Malaysia, Indonesia	Mudflats of intertidal zone in estuaries with mangrove
Scoletoma heteropoda	Chrori	Japan, Southern Sakhalin, Yellow Sea	Mud and Sandflats of intertidal to subtidal zone
Halla okudai	Tai-mushi	Japan, China, Malaysia, Southern Austraria	Sandflats of intertidal to shallow subtidal zone
Pseudopotamilla occelata	Erako	Northern Japan, Pacific ocean	Surface of rocks of intertidal to subtidal zone
Hydroides ezoensis	Pipe-mushi	Japan, Russia	Surface of rocks of intertidal to subtidal zone
Urechis unicinctus	Kouji, Super Kouji, Yu-mushi	China, Korea, Japan	Mud and Sandflats of intertidal to subtidal zone
Sipunculus nudus	BB Worm	Atlantic, Pacific and Indian Oceans, Mediterranean and Red Seas	Sand flats intertidal to shallow subtidal zone

Hediste diadroma Sato and Nakashima, 2003

This species is a nereid worm, which was known as *Neanthes japonica* (Izuka, 1908) (e.g., Izuka, 1908; Imajima, 1972, 1996), but then described as a new species by Sato and Nakashima (2003). This species is found in the intertidal muddy and sandy sediments of estuaries of Japan and China (Sato and Nakashima, 2003). It is harvested during late November to March (until reproductive swarming occurs) around Kojima (Okamaya) under the local market name ‘Mizu-gokai’, meaning water worm.

Kinbergonuphis enoshimaensis Imajima, 1986

This species is a onuphid worm that lives in sandy sediments of the intertidal zone of Central and Western Japan (Enoshima and Amakusa) (Imajima,1986, 2001). A limited number are harvested from sandy coasts of the open sea around Tohashi, Aichi, central Japan under the local market name 'Iso-mushi', meaning beach worm. Wholesalers reported that bait collectors attract this worm by scattering olfactory stimulants such as fish and shellfish on sand, and then by digging with a shovel.

Pseudopotamilla occelata Moore, 1905

This species is a sabellid worm found on the surface of rocks of the intertidal zone of Northern Japan and the Pacific Ocean (Uchida, 1992). Limited numbers are harvested in Miyagi, northern Japan under the local market name 'Erako', meaning branchiae worm.

Hydroides ezoensis Okuda, 1934

This species is a serpulid worm that lives on the surface of rocks, shells, the holdfasts of kelp, and other substrata in Japan and Russia (Imajima, 1976, 1996). Limited numbers are harvested at Osaka, western Japan under the local market name 'Pipe-mushi', meaning pipe worm. Wholesalers reported that this species is used as bait for black seabream, *Acanthopagrus schlegelii* to hook several calcareous tubes which worms are entering. *Hydroides ezoensis* has been introduced to British waters, suggesting that this species was transported by shipping from the north-west Pacific, perhaps from Japan (Thorp *et al.*, 1987).

2) Worms supplied from native and non-native populations

Marphysa cf. *iwamushi* Izuka, 1907

The eunicid worm, *Marphysa iwamushi* was described by Izuka, 1907, but then synonymized with *Marphysa sanguinea* by Imajima and Hartman, 1964 (Miura, 1977; Imajima, 2007). This worm lives in sandy and rocky sediments from the intertidal to subtidal (Izuka, 1912; Imajima, 2007). Recently, Hutchings and Karageorgopoulos (2003) redescribed *Marphysa sanguinea* using a specimen collected in England as the type locality. Subsequent taxonomic revisions of the *Marphysa sanguinea* group have been done from different parts of the world (Lewis and Karageorgopoulos, 2008; Glasby and Hutchings, 2010). Lewis and Karageorgopoulos (2008) reported that there is sufficient genetic differentiation between the geographically separated populations of Australia, England, Japan, Portugal, and South Africa, suggesting that *Marphysa sanguinea* does not occur in Japan. More recently, Taru (2013) recognized *Marphysa iwamushi* as a valid species. This worm similar to Imajima's (2007) description whose subacicular chaetae comprise compound spingerous chaetae only. This worm has been imported from Korea since 1969, although the main source country has shifted to China (Saito *et al.*, 2011). A small amount of the worm is harvested in Japan under the local market names 'Iwa-mushi' and 'Hon-mushi', meaning rock worm and genuine worm, respectively.

Halla okudai Imajima, 1967

This species is an oenonid worm that lives in sandy sediments of the intertidal to shallow subtidal in Japan, Malaysia, and Southern Australia (Okuda, 1933; Imajima, 1967; Idris and Arshad, 2013). Limited numbers of this species have been harvested in Hiroshima, western Japan under the local market name 'Tai-mushi', meaning bream worm. Since 2004, this worm has been imported from Fujan, southern China. Wholesalers mentioned that *Halla okudai* is the most effective worm of all bait worms for red seabream, *Pagrus major*, but that supplies are limited. Therefore, the market price is very high (6500 yen/100 g).

Halla okudai is a carnivorous worm feeding on bivalves, especially the manila clam, *Ruditapes phillipinarum* (Saito et al., 2004). Recently, there are concerns that there has been a collapse of the Japanese population, because production of the clam decreased drastically in Japan (The Japanese Association of Benthology, 2012).

Urechis unicinctus (von Drasche, 1881)

This species is a urechid spoon worm, which is found in muddy and sandy sediments of the intertidal to shallow depths of China, Korea, and Japan. It has been harvested from the Seto Inland Sea, western Japan under the local market names 'Kouji', 'Super Kouji', and 'Yu-mushi', which all mean good bait (Saito *et al.*, 2011). This worm has been imported from the Shandong Peninsula, China since 1996. This species is also consumed by humans in China, Korea, and Japan (Hokkaido) (Nishikawa, 1992).

3) Worms supplied from non-native populations

Perinereis linea (Treadwell, 1936)

This species is a nereid worm, which has been imported from Korea since 1969, although the main source country is now China (Hayashi, 2001). It was formerly recognized as *Perinereis aibuhitensis* Grube, 1878 (e.g. Imajima, 1996), but was synonymized with *Perinereis linea* by Arias et al. (2013). This species is found in silty sediments in the upper littoral zone of estuaries and coastal areas of China and Korea (Choi and Lee, 1997; Arias *et al.*, 2013). Wholesalers mentioned that *Perinereis linea* has been mainly harvested from the Yellow Sea population (worms have a greenish body color) in summer and the East China Sea population (worms have a yellowish body color) in winter. In addition, both populations have been cultured recently in the South China Sea (Hainan) under the local market names 'Super Ao-mushi' and 'Aka-isome', meaning excellent blue worm and red worm, respectively.

Alitta virens (Sars, 1835)

This species is a nereid worm, which lives in muddy and sandy sediment of the intertidal to subtidal in estuaries and the coasts of the North Sea, Northern Atlantic, and Pacific Oceans (Khlebovich, 1996; Bakken and Wilson, 2005). The aquaculture of this species in European countries began in 1979 (Olive, 1994). In Japan, this cultured worm has been imported from The Netherlands since 1994 under the local market name 'Ao-kogane', meaning blue gold. In Japan, a common name 'Jya-

mushi', meaning snake worm, was known as *Neanthes virens* (e.g., Imajima, 1972, 1996) , but then synonymized with *Alitta brandti* Malmgren, 1865 by Khlebovich (1996).

Nectoneanthes uchiwa Sato, 2013

This species is a nereid worm, which was formerly recognized as *Nectoneanthes oxypoda sensu* Imajima, 1972 (e.g., Imajima, 1972, 1996), but then described as a new species by Sato (2013). This species inhabits muddy sediments in the intertidal or shallow subtidal areas (up to 20 m depth) of estuarine embayments of Western Japan (Seto Inland Sea, Ariake Sea, and Shiranui Sea), Korea, and China (Sato, 2013). It was once harvested in the Seto Inland Sea, western Japan (Okuda, 1933). This species has been imported from China since the 1980s (Wu *et al*., 1985), and we obtained it in Osaka, western Japan under the local market name 'Aka-kogane', meaning red gold.

Namalycastis rhodochorde Glasby, Miura and Nishi, 2007

This species a nereid worm, which lives in mud banks and mudflats of estuaries with mangroves in South-east Asia including the Mekong Delta (Vietnam), West Kalimantan (Indonesia), and Sabah (Malaysia). It has been imported from Vietnam into Japan since 1993 (Glasby *et al*., 2007). We obtained this worm (online order) from Sendai, northern Japan under the local market name 'Super Cordelle '.

Glycera nicobarica Grube, 1868

This species is a glycerid worm that lives in intertidal to subtidal sandy sediments of Japan, the East China Sea, Southern Pacific, and Indian Oceans (Imajima, 2007). Glycerid worms (probably *Glycera americana*) were once harvested in the Seto Inland Sea, western Japan (Okuda, 1933). *Glycera nicobarica* has been imported from China since 2010 under the local market names 'Chi-mushi' and 'Shro-chirori', meaning blood worm and white proboscis worm, respectively.

Diopatra sugokai Izuka, 1907

This species is an onuphid worm, which inhabits intertidal to subtidal sandy sediments of estuaries and coastal waters of Malaysia, Thailand, China, Taiwan, and Japan (Choe, 1960; Paxton, 1998; Imajima, 2001). It was once harvested in Matsushima Bay, Tokyo Bay, Ise Bay, the Seto Inland Sea, and the Ariake Sea, Japan (Choe, 1960). Recently, it has been imported from China under the local market name 'Fucro-mushi', meaning tube worm.

Marphysa cf. *tamurai* Okuda, 1934

This eunicid worm, sold under the local market name 'Honsa-mushi', meaning genuine sand worm, has been imported from China since 2008. According to the Key to Indo-west Pacific *Marphysa* species (Glasby and Hutchings, 2010), this worm resembles *Marphysa tamurai* whose prostomium is sub-conical and buccal lips are separated by a faint notch. *Marphysa tamurai* is found in muddy and sandy sediments of the intertidal zone of Central and Western Japan (Ise Bay and Onomichi) (Okuda, 1934, 1938). However, there is a lack of recent information on the habitat of this species in Japan.

Marphysa cf. *mossambica* (Peters, 1854)

This eunicid worm, sold under the local market name 'Straw-mushi', meaning worm entering a straw tube, has been imported from Indonesia since 1995. This worm seems to belong to the Mossambica-group whose subacicular chaetae comprise limbate chaetae only (Glasby and Hutchings, 2010). According to observations by Idris (Idris, pers comm. 2013), this worm is similar to *Marphysa* cf. *mossambica* from Malaysia, and is most probably a new species (Idris and Arshad, 2013). This species is found in mangroves and mud flats along the west coast of the Malaysian peninsula (Idris and Arshad, 2013).

Scoletoma heteropoda (Marenzeller, 1879)

This species is a lumbrinerid worm, which is found in intertidal and subtidal muddy and sandy sediments of Japan, Southern Sakhalin, and the Yellow Sea (Imajima and Higuchi, 1975; Imajima, 2001). It was once harvested in the Seto Inland Sea and the Ariake Sea, western Japan (Saito *et al*., 2011). Recently, it has been imported from China under the local market name 'Chirori'.

Sipunculus nudus Linnaeus, 1766

This species is a sipunculid peanut worm, which lives in intertidal to shallow subtidal sandy sediments of the Atlantic, Pacific, and Indian Oceans and Mediterranean and Red Seas (Culter *et al*., 1984; Nishikawa, 1992). It has been imported from China since 2010 under the local market name 'BB Worm'. This species is edible and consumed in Micronesia, the Philippines, and China (Nishikawa, 1992; Tsuji, 2007).

Survival experiments

The nereid worms had a wide salinity tolerance range of 5–35 psu in *Alitta virens,* 5–30 psu in *Namalycastis rhodochorde,* and 10–35 psu in *Perinereis linea*. *Marphysa* cf. *mossambica* showed a wider tolerance (15–35 psu) than *Marphysa* cf. *iwamush* (20–35 psu). *Halla okudai* did not survive below 25 psu (Fig. 2).

The temperature tolerances of worms from temperate areas had a range of 5–30 °C in *Alitta virens, Perinereis linea*, *Glycera nicobarica*, *Marphysa* cf. *iwamushi*, and *Scoletoma heteropoda*, and 10–35 °C in *Halla okudai*. The tropical species, *Namalycastis rhodochorde* and *Marphysa* cf. *mossambica*, did not survive below 20 °C (Fig. 3).

Discussion

In Japan, bait worms were once collected mainly from the intertidal zone of the Seto Inland Sea and Ise Bay (Okuda, 1933, 1938). However, large parts of the sandy and muddy intertidal flats of the Japanese coast, including areas used by bait collectors, have disappeared because of anthropogenic coastal developments (e.g., reclamation, seawall construction) (Sato, 2010). To satisfy the demand of Japanese anglers, two species of nereid and eunicid worm have been imported from Korea since 1969, although the main source country shifted to China after the 1990s with an annual supply of approximately 1000 t and an increasing number of bait species (Hayashi, 2001).

In this study, a total of 25 types of live bait worms were obtained in Japan, and we were able to identify 16 species of

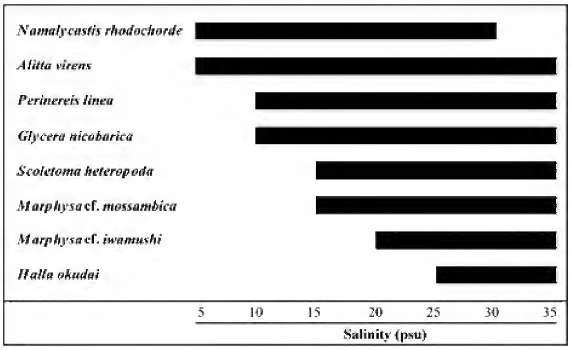

Fig. 2. Schematic representation of inhabitable salinity range of imported worms. The inhabitable range of salinity was evaluated from the 100 % survival of individuals after 5 days.

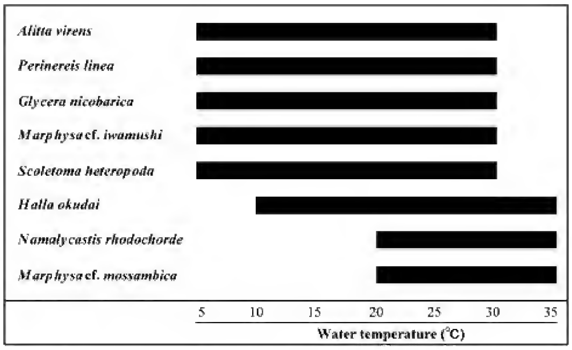

Fig. 3. Schematic representation of inhabitable temperature range of imported worms. The inhabitable range of temperature was evaluated from the 100 % survival of individuals after 5 days.

polychaete, 1 species of echiuran, and 1 species of sipunculid. These were divided into three groups according to their country of origin: 1) worms supplied from native populations, five species (*Perinereis wilsoni*, *Hediste diadroma*, *Kinbergonuphis enoshimaensis*, *Pseudopotamilla occelata*, and *Hydroides ezoensis*), 2) worms supplied from both native and non-native populations, three species (*Marphysa* cf. *iwamushi*, *Halla okudai*, and *Urechis unicinctus*), and 3) worms supplied from non-native populations, 10 species (*Perinereis linea*, *Alitta virens*, *Nectoneanthes uchiwa*, *Namalycastis rhodochorde*, *Glycera nicobarica*, *Diopatra sugokai*, *Marphysa* cf. *tamurai*, *Marphysa* cf. *mossambica*, *Scoletoma heteropoda*, and *Sipunculus nudus*). Other countries have also been reported to import bait worms. In the United States, California imports two species from South Korea (*Perinereis linea*) and Vietnam (*Namalycastis rhodochorde*) (Cohen, 2012). Likewise, Spain and Portugal import three species, *Perinereis linea* from China, *Sipunculus nudus* from Vietnam, and *Glycera dibranchiata* from the USA (Costa *et al.*, 2006). These reports indicate that Japan imports more bait species than other countries. It seems that the difference in the number of imported species is caused by the presence of domestic fishing grounds in Europe and the United States (Cunha et al., 2005; Sypitkowski et al., 2010).

The bait industry is considered as an important vector of invasive species (Weigle *et al.*, 2005; Haska *et al.*, 2012; Kilian *et al.*, 2012). Kilian *et al.* (2012) reported that 65% of anglers released their unused bait into the water at the end of a fishing trip. Indeed, Nishi and Kato (2004) reported that *Perinereis linea* was discarded in Tokyo Bay (Yokohama, Japan) by fishermen. In Japan, among the bait worms, nereid worms are inexpensive (market price of *Perinereis linea* is approximately 10% of *Halla okudai*) and are mass-supplied items. Consequently, they tend to be discarded into the water. Our research revealed that *Alitta virens*, *Namalycastis rhodochorde*, and *Marphysa* cf. *mossambica* are considered to be non-native species as their native distributional area is outside of Japan. In addition, *Nectoneanthes uchiwa* and *Halla okudai* are listed as endangered species in Japan (The Japanese association of benthology, 2012). Hence, the import of bait worms may increase the risk of accidental introduction of non-native species and change the distribution pattern of rare species.

In this study, the nereid worms *Alitta virens* and *Perinereis linea*, which inhabit boreal and temperate zones have wide salinity and temperature tolerances of 5 or 10 to 35 psu, and 5–30 °C, respectively. *Alitta virens* inhabits the White Sea, whose temperature and salinity varies during the year from 0 to 1 °C in winter up to 20 °C in summer, and from 22 to 26 psu during the year to 0–5 psu for several days during the spring ice melt (Ushakova and Sarantchova, 2004). In the Yellow Sea, *Perinereis linea* inhabits silty sediments of the upper littoral zone of estuaries where sediment temperature is 3.3–26.6 °C and salinity 28.0–29.6 psu (Choi and Lee, 1997). More recently, an established population of the exotic worm *Perinereis linea* was reported from the Mar Menor lagoon, Mediterranean Sea where salinities of 42–47 ppt and temperatures of 10.8–31.5 °C have been recorded (Arias *et al.*, 2013). These data indicate that both worms can survive a range of temperatures and salinities.

Our experiments showed that two tropical worms had a salinity tolerance with a range of 5–30 psu for *Namalycastis rhodochorde*, and 15–35 psu for *Marphysa* cf. *mossambica*. Both species could not survive in water temperatures below 20 °C. Glasby *et al.* (2007) reported that *Namalycastis rhodochorde* was distributed throughout South-east Asia, where it inhabits mud banks and mudflats of estuaries and rivers from full seawater to almost freshwater. The mangrove palm, *Nypa fruticans*, is present as far south as northern Australia (Northern Territory and North-east Queensland) and northward to southern Japan (Yaeyama Islands). Therefore, there is a possibility that *Namalycastis rhodochorde* also occurs there, or if not native to these areas, could become established if introduced. It is possible for anglers in southern Japan to obtain *Namalycastis rhodochorde* and *Marphysa* cf. *mossambica*, because they are sold online. Therefore, detailed monitoring of their establishment should be undertaken.

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Shallow-water polychaete assemblages in the northwestern Mediterranean Sea and its possible use in the evaluation of good environmental state

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Abstract

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Forty-four shore-normal transects along the Northwestern Mediterranean coast between the mouth of the Rhône River (France) and Valencia City (Spain) were sampled during the REDIT-I (September 1998, [R1]) and REDIT-II (December 1999, [R2]) campaigns. Polychaete distribution patterns on shallow littoral fine sands (10 to 50 m water depths) were analyzed at a regional scale. A total of 359 species of polychaetes were identified which represents 38% of all polychaete species recorded in the western Mediterranean. Four main soft-bottom communities were identified from the samples in the area: Littoral Fine Sands, Littoral Sandy Mud, Terrigenous Coastal Mud and Detritic Sand. Predominantly sandy environments were characterized by *Ditrupa arietina* and *Owenia fusiformis* while *Lumbrineris latreilli*, *Hilbigneris gracilis* and *Sternaspis scutata* were numerically dominant in muddy environments. Biological diversity assessments at different temporal and spatial scales are required by the European Marine Strategy Framework Directive (2008/56/EC) in accordance with criteria and methodological standards of Good Environmental Status (GEnS). Selected indicators for descriptors are explored based on this mesoscale assessment.

Keywords

Polychaeta. Northwestern Mediterranean, Marine Strategy Framework Directive, GEnS. Infauna.

Introduction

The assessment of biological diversity at different temporal and spatial scales is a prerequisite when criteria and methodological standards on Good Environmental Status of marine waters (GEnS, following Mee et al., 2008) need to be evaluated following the Marine Strategy Framework Directive (MSFD) (2008/56/EC) (European Commission, 2008). For these assessments, ecosystem integrity as well as particular pressures requiring management responses, need to be understood across biogeographic regions (Cochrane et al., 2010). Following the recommendations of the MSFD, a suitable set of European ecological assessment areas should be defined to analyse habitat

and community distributions and condition. Initial assessments have been recently carried out by member states. This knowledge is basic for cooperation in planning future coastal and marine conservation and uses, as well as for further implementation of the MSFD. Although indicators of GEnS are required by the Directive at national, ecoregional or sub-regional inside subnational economic exclusive zone scales, its application at other geographical spatial scales should be also possible and even advisable in marine management (Sardá et al., in press).

During 1998–99, cooperation between French and Spanish scientific institutions was initiated to assess the biological diversity from shallow soft-bottom environments in the Gulf of Lions and the northern Mediterranean Spanish coast (10 to 50 m water depths). The main aim of the study was to describe the distributions of benthic species present in the region as well as the range of its existing benthic communities. This region comprises around 2000 km of coastal fringe and can be considered as a suitable area for assessment and implementation of the MSFD because of its size, social and ecological importance and existing scientific knowledge.

The Gulf of Lions was the departure point for the pioneer biological description of soft-bottom communities in the Mediterranean (Pérès and Picard, 1964; Picard, 1965; Guille, 1970, 1971; Massé, 1972; Bellan and Bourcier, 1984). Some decades later, the distribution, composition and ecological quality of the benthic macroinfauna in the Gulf of Lions were reassessed by Grémare et al., (1998a; 1998b), and more recently by Labrune et al., (2006a, 2006b, 2007, 2008). The unification of the terminology for the description of the soft-bottom communities observed in the Gulf of Lions by these authors was one of the main results of the REDIT-I Program. Three main communities corresponding with historical community classification data (Pérès and Picard, 1964; Picard, 1965; Guille, 1970) were detected: Littoral Fine Sands (LFS), Littoral Sandy Mud (LSM), and Terrigenous Coastal Mud (TCM).

Polychaetes are one of the dominant and characteristic groups of soft-bottom communities (Knox, 1977; Coll et al., 2010). It has been shown that, in most cases, polychaetes constitute a good surrogate for describing the functioning of the entire benthic community (Giangrande et al., 2005) and aid assessment of environmental condition. The numerical dominance, multiple life history traits and relatively large knowledge base about polychaetes call for inclusion as GEnS benthic indicators and offer a means to understand the mechanisms governing community dynamics.

In Europe, the recently introduced Marine Strategy Framework Directive (MSFD) seeks to implement the ecosystem approach to marine management to deliver protection of marine ecosystems while at the same time recognising the needs of society to benefit from marine resources allowing its sustainable use. The main objective of MSFD is to achieve GEnS for its marine waters by 2020 and the resources upon which marine-related economic and social activities depend through an integrated ecosystem-based approach. The approach promotes a holistic view on management by ensuring sustainable use of the seas; providing safe, clean, healthy and productive marine waters (Browman et al., 2004; Borja et al., 2011). The MSFD establishes European Marine Regions on the basis of geographical and environmental criteria. Each member state, in cooperation with other member states and non-EU countries within a marine region, are required to develop strategies for their marine waters. The marine strategies to be developed by each member state must contain a detailed assessment of the state of the environment, a definition of GEnS at regional level and the establishment of clear environmental targets and monitoring programs to reach and maintain such GEnS.

In this paper we introduce new data from the North-western Mediterranean coast of Spain to the previous analysed Gulf of Lions region (Labrune *et al.*, 2006a, 2006b, 2007, 2008). Using all this data, the aim of the present study is to describe the distributional range of soft-bottom communities and their associated polychaete species while addressing the suitability of using particular indicators derived from this analysis for the MSFD, especially for Biodiversity descriptor of GEnS but also to explore the suitability for further descriptors of the Directive.

Material and methods

Sampling and laboratory procedures

Benthic samples were obtained at 44 transects perpendicular to the shore, extending from the mouth of the Rhône River (France) (43°19'55"N, 4°44'56"E at the 10 m station) south to Valencia city (Spain) (39°28'23"N, 0°18'30"E at the 10 m station) (Figure 1). At each transect three macroinfaunal samples were taken from each of five stations at 10, 20, 30, 40 and 50 m water depths. An additional sample was taken for sedimentological analyses. Samples were collected during the REDIT-I and REDIT-II oceanographic campaigns. The REDIT-I campaign (September 1998) was carried out from the mouth of the Rhône River to the French-Spanish border on board the N.O. Georges Petit. The REDIT-II campaign (December 1999) was carried out from the French-Spanish border to the vicinity of the city of Valencia on board the N.O. Tethys. A total of 220 stations were sampled. Sampling failed at 20 stations (10 m samples at R1O, R1P, R2C, R2D, R2F, R2I, and R2S; 20 m samples at R2I and R2S; 30 m samples at R2I; 40 m samples at R1O, R1P, R1Q, R1R and R1S; and 50 m samples at R1O, R1P, R1Q, R1R and R1S).

Samples were collected using a 0.1 m^2 van Veen grab and sieved on board using a 1 mm mesh. The mesh residue was fixed in 5% formaldehyde buffered in seawater. As described in previous works (Labrune et al., 2006a, 2006b, 2007, 2008), this mesh was selected to enable a comparison with previous works carried out in the region. Grabs with penetration lower than 15 cm were rejected and all samples (with none, few or significant algal or detrital material) were treated in the same way. In the laboratory, samples were sorted under a dissecting microscope and all faunal groups separated. Polychaetes were later identified to the lowest practical taxonomic level and counted. Gil (2011) was used as reference work for identification. Unidentified species were only considered when they were sufficiently complete, mature and distinct from identified species. Data analyses were carried out on data pooled over the three replicated sampling units (Ellingsen, 2001). Individual polychaete species biomass was determined as wet weight

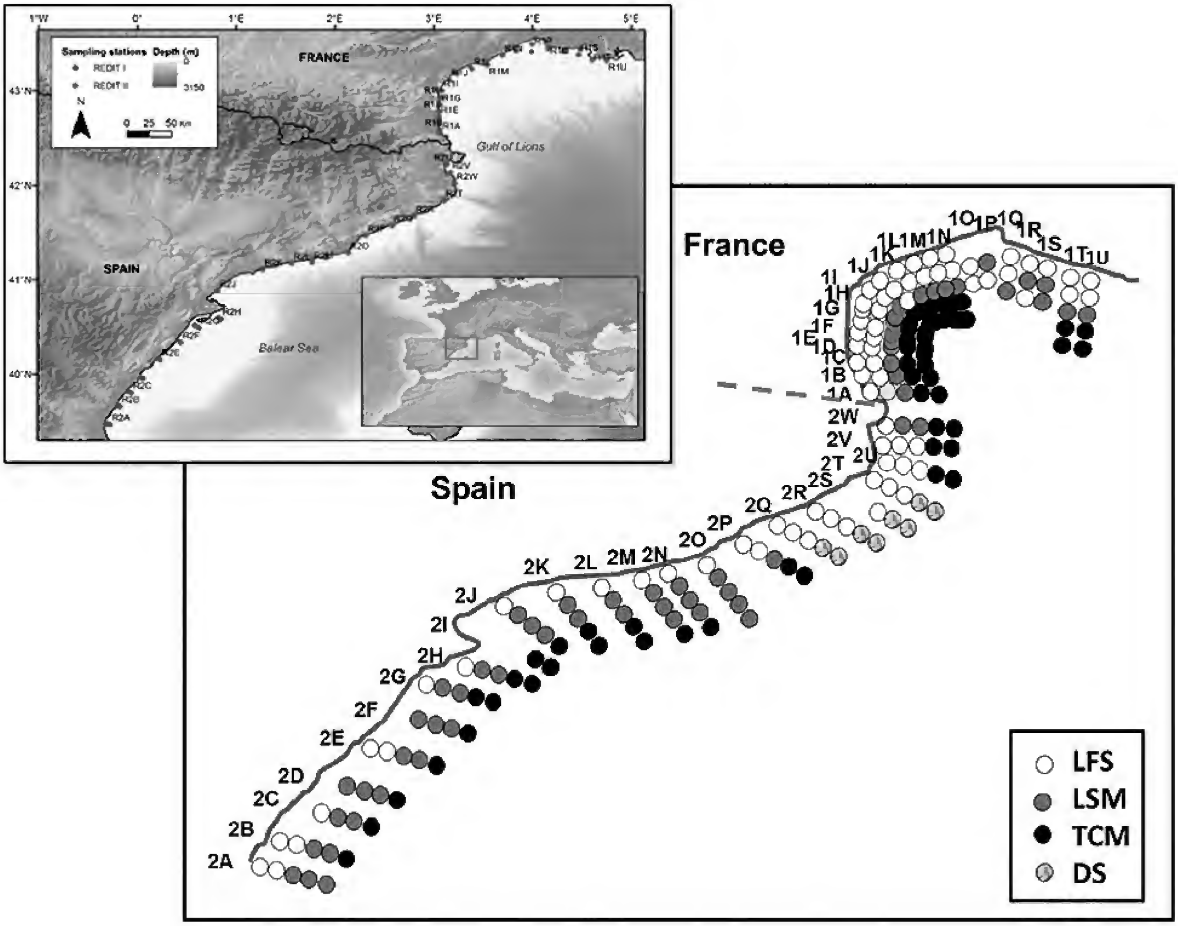

Figure 1. (Upper left graph) Map of the studied zone. Blue circles represent sampled stations from the Gulf of Lions and red circles from the Northern Mediterranean Spanish coast. (Lower graph) Schematic diagram showing the distribution of the four studied communities in the mesoscale studied area

(avoiding presence of water outside the animal when weighting it) to avoid destruction of the collected material except for two nominal species *Ditrupa arietina* (O.F. Müller, 1776) and *Owenia fusiformis* Delle Chiaje, 1844. For these two species we used regressions of width vs. dry weight to convert width measurements to biomass following Sardá et al. (1999).

D. arietina: $DW_{Da} = 0.4522\ (d_{Da})^{3.992}$

O. fusiformis: $DW_{Of} = 0.8434\ (wt_{Of})^{2.177}$

where DW_{Da} and DW_{Of} are dry weights of both species in mg and (d_{Da}) is diameter aperture of the *D. arietina* tube, in mm, and (wt_{Of}) is the maximum width of the tube of *O. fusiformis* in mm. For comparative purposes biomass data given in this paper is expressed in dry weight using the conversion factor of dry weight = 17.6% of wet weight calculated for polychaete species (Rumohr et al., 1987).

Bionomic data are given as means for each measured parameter per station (biological: abundance, biomass, richness, diversity; sedimentological: D50, silt/clay%) for each identified assemblage. Basic sediment texture features that might be correlated with assemblages were derived from granulometric analysis conducted on fresh sediment using a Malvern® Mastersizer 2000 laser microgranulometer.

Data analysis and cartographical work

Analysis of species data for the classification of the polychaete assemblages was performed on reduced sets of species in order to limit noise introduced by less common species and its associated distorting effects in the analytical work. Species present in less than 10% of the obtained samples were excluded; rare species usually have little meaning for the description of benthic communities and their omission does not affect community interpretation.

Table-1. List of the eleven qualitative descriptors of Good Environmental Status (GEnS) according to the Marine Strategy Framework Directive. In parentheses is indicated the task group study for the introduction of indicators

1.	Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions. (Cochrane et al., 2010)
2.	Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems. (Olenin et al., 2010).
3.	Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock. (Piet et al., 2010).
4.	All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity. (Rogers et al., 2010).
5.	Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters. (Ferreira *et al.*, 2010).
6.	Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected. (Rice et al., 2010).
7.	Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.
8.	Concentrations of contaminants are at levels not giving rise to pollution effects. (Law et al., 2010).
9.	Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards. (Swartenbroux et al., 2010).
10.	Properties and quantities of marine litter do not cause harm to the coastal and marine environment. (Galgani et al., 2010)
11.	Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment. (Tasker et al., 2010).

In this paper we are presenting and using the two obtained sub-regional clusters (France and Spain) following the initial mandate of the MSFD to work on subnational economic exclusive zone regional scales. Multivariate analyses were performed in order to elucidate similarities. Polychaete assemblages were constructed from cluster analysis corresponding to similarities of approximately 25% (Bray Curtis similarity index, average link grouping). Densities were square-root transformed to limit the influence of the most dominant taxa (Clarke and Warwick, 1994). The taxa most responsible for similarities within each cluster of stations or for dissimilarities between clusters of stations were identified using the SIMPER procedure. The Shannon-Wiener information index (H', log *e*) was used as a measure of diversity. All multivariate analyses were carried out using the Primer© 6 software package (version 6.1.13) (Clarke and Gorley, 2006). The Benthic Quality Index (BQI) (Rosenberg et al., 2004) was computed as an estimate of integrity.

Benthic production estimates were based on biomass data. In order to rank the most important polychaete species contributing to the productivity of the region, we estimated secondary production using the allometric equation developed by Brey (1990):

$$P = (B/A)^{0.73} * A$$

where *A* is density, *B* is biomass, *B/A* is mean individual biomass and *0.73* is the average exponent of the regression of annual production on body size for macrobenthic invertebrates. This indirect method is based on the use of empirical relationships and yields the secondary production of all species within a community.

The geographical extent of the five identified communities was determined. The study area was defined as the union of a convex hull polygon containing all samples and the relief area of bathymetric data (Catalano-Balearic Sea – Bathymetric chart, 2005, www.icm.csic.es/geo/gma/MCB) from 5 to 55 m contours. The study area was divided into a regular grid of 500 x 500 m. The presence or absence of each community was identified at each station. Inverse distance weighting (IDW, Cressie 1993) was used to interpolate the presence or absence of each community at non-sampled grid cells and estimate community areal coverage. All data were re-projected to the projection system ETRS89 LAEA. Data analysis was conducted using the 'idw' function (ie., setting an inverse distance weighting power = 20) from the 'gstat' package (Pebesma, 2004) and the R software (http://www.r-project.org).

Utility of data as indicators for qualitative descriptors of GEnS

The MSFD (EC, 2008) describes GEnS based on eleven qualitative descriptors and indicators selected from those published by each descriptor task force (Table 1). Recently, both France and Spain presented their initial assessment for the Mediterranean and the Levantine-Balearic regions. Here, we assess the suitability of the use of the REDIT campaigns data in relation with the qualitative descriptor of GEnS to contribute to the improvement of the knowledge of these areas.

Table 2. Mean benthic parameters for the different assemblages identified in the REDIT Campaigns. All values are computed as the mean of each considered parameter (D50, %silt/clay, abundance, biomass, richness, and diversity) per station for all the stations included in the same group except for total richness where the accumulated number of species found in all stations of the group is given. D50 computes the mean grain size for each identified assemblage. (Da)* LFS Spanish assemblage with high numbers of *Ditrupa arietina*.

	Assemblages							
	LFS			LSM		TCM		DS
	France	Spain	Spain (Da)*	France	Spain	France	Spain	Spain
Granulometry								
D50 (um)	145.8	126.1	299.8	86.5	99.6	21.2	25.1	355.0
Silt/Clay content(%)	8.7	5.8	11.7	29.5	56.2	79	77.3	18.9
Biological parameters								
Abundance (ind sq m)	1074	646	1006	473	719	179	468	896
Biomass (mg dw sq m)	1031	385	979	112	1030	184	375	372
Total Richness (#)	105	160	154	85	249	85	138	123
Average Richness (#)	20	26	35	16	32	18	18	36
Diversity (H')	1.58	1.87	2.58	1.95	2.66	2.31	2.27	2.95

Indicator used in the assessment of some of the Good Environmental Status descriptors using data for the REDIT mesoscale assessment (sq m equals to ind m^{-2}).

Results

Assemblage classification and key species

About 35,000 polychaete and sipunculan specimens were identified representing 359 species. More species were found in the Spanish region (325 species) than the French region (175 species), but the area covered by the Spanish campaign was also larger. In the case of polychaetes they constitute 38% of all known Western Mediterranean species (Gil, 2011). In the French region, three main polychaete assemblages were identified based on a 25% similarity level (fig. 2, upper graph). In the Spanish region, based on the same 25% similarity level, two assemblages were identified, both of them with clear sub-clusters (fig. 2, lower graph). The distributions of these assemblages were related to depth and sedimentological parameters. Mean sediment grain size decreased with depth and increasing percentage of silt and clay; only deep stations off rocky shores in the Costa Brava showed a different pattern, forming detritic sand bottoms. Other variables such as abundance, biomass, and diversity are highly correlated to the presence of two, shallow-dwelling species located in sandy environments *Ditrupa arietina* and *Owenia fusiformis*. Sedimentary and biological characteristics of the proposed assemblages are presented in Table 2.

Three main clusters were identified in the French region (fig. 2, upper graph). Cluster I was comprised of 10 and 20 m stations associated with Littoral Fine Sands (LFS sensu Labrune et al., 2007). Cluster II grouped 30 m stations with a higher content of fines (LSM sensu Labrune et al., 2007) and could be separated into two sub-clusters based on geographical considerations (Labrune et al., 2007). Finally, Cluster III gathered 40 and 50 m stations from muddy sediments (TCM sensu Labrune et al., 2007).

In the Spanish region two main clusters were delineated; Cluster I consisted of stations associated with Littoral Fine Sands (LFS), but could be further divided into two sub-clusters (Ia and Ib) due to the presence or absence of the polychaete *D. arietina* respectively. In the sub-cluster Ib, a separate set of stations of the LSM community can be seen with the common presence of *D. arietina* in the samples. Cluster II included the rest of the stations with two sub-clusters: sub-cluster IIa incorporated most of the 50 m deep stations on muddy sediments (TCM), and sub-cluster IIb was composed of a more heterogeneous set of samples, both in depth (with a lower percentage of fines) and in species composition, and were more similar to the LFS assemblage. One particular group of stations within sub-cluster IIb (with asterisk in fig. 2, lower graph) was also differentiated by deeper stations, but with sedimentological composition of medium sands and a smaller (18.9%) percentage of fines. These stations could not be incorporated into any of the previously named assemblages and were assigned to a Detritic Sand (DS) community.

The most abundant species for each of the assemblages are shown in Table 3. The density of the first six species accounted for 66% of the total average density in the Littoral Fine Sands (LFS) assemblages, 53.9% in the Littoral Sandy Mud (LSM) assemblages, 54.7% in the Terrigenous Coastal Mud (TCM) assemblages, and 53.7% in the Detritic Sand environment (DS) off Costa Brava.

LFS assemblages were characterized by high densities (79% in the case of the French region) of two species, *D. arietina* and *O. fusiformis*. The presence of *D. arietina* is the determining factor that separated different assemblages in this community (Table 3). Both species were more abundant in the Gulf of Lions than in the northern Mediterranean Spanish coast resulting in a more homogeneous composition in this area.

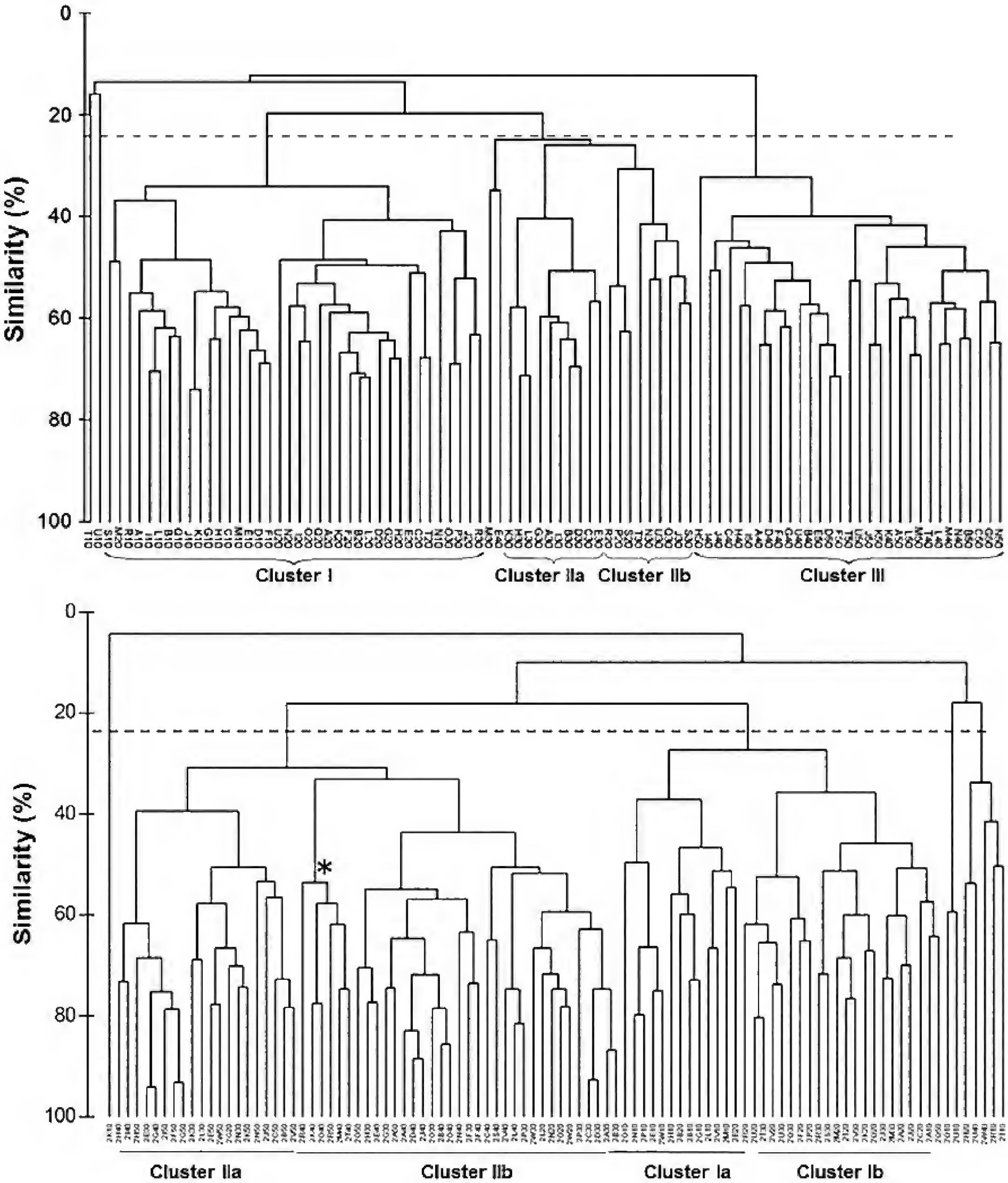

Figure 2. Cluster analysis of polychaete fauna for the Gulf of Lions region (France; upper graph) and the Northern Mediterranean Spanish coast (Spain; lower graph). Asterisk observed in lower graph indicates stations associated with the Detritic Sand Community (DS)

Table 3. Six most dominant species of each assemblage identified during the present study and its average density (ind m^{-2}).

LITTORAL FINE SAND Community (LFS)

FRANCE				**SPAIN**			
LFS with *Ditrupa*				LFS with *Ditrupa*		LFS without *Ditrupa*	
Ditrupa arietina	616			*Ditrupa arietina*	302	*Owenia fusiformis*	129
Owenia fusiformis	233			*Eunereis longissima*	27	*Spiochaetopterus costarum*	50
Aponuphis bilineata	42			*Aponuphis bilineata*	25	*Chone duneri*	35
Chone duneri	30			*Mediomastus fragilis*	21	*Notomastus latericeus*	30
Scoletoma impatiens	24			*Galathowenia oculata*	21	*Pseudopolydora paucibranchiata*	25
Lumbrineris latreilli	21			*Protodorvillea kefersteini*	21	*Galathowenia oculata*	23

LITTORAL SANDY MUD Community (LSM)

FRANCE				**SPAIN**			
LSM west Cap ‘Agde		LSM east Cap ‘Agde		LSM			
Lumbrineris latreilli	171	*Lumbrineris latreilli*	91	*Monticellina heterochaeta*	82		
Ditrupa arietina	100	*Nephtys hombergii*	18	*Hilbigneris gracilis*	70		
Goniada emerita	36	*Mediomastus fragilis*	15	*Sternaspis scutata*	30		
Scoletoma impatiens	34	*Glycera unicornis*	14	*Aponuphis bilineata*	27		
Hilbigneris gracilis	30	*Notomastus latericeus*	11	*Notomastus latericeus*	24		
Laonice bahusiensis	21	*Scoletoma impatiens*	7	*Lumbrineris latreilli*	24		

TERRIGENOUS COASTAL MUD Community (TCM) and DETRITIC SAND Community (DS)

FRANCE				**SPAIN**			
TCM				TCM		DS	
Lumbrineris latreilli	41			*Hilbigneris gracilis*	87	*Aspidosiphon muelleri*	220
Sternaspis scutata	25			*Monticellina heterochaeta*	72	*Sphaerosyllis taylori*	83
Heteromastus filiformis	12			*Prionospio fallax*	34	*Pisione remota*	73
Nephtys incisa	11			*Sternaspis scutata*	29	*Kefersteinia cirrata*	58
Abyssoninoe hibernica	7			*Cirrophorus branchiatus*	18	*Ditrupa arietina*	43
Glycera unicornis	6			*Galathowenia oculata*	16	*Heteromastus filiformis*	34

LSM assemblages were the most diverse group. In the French region, sub group Ia was identified north of Cap ‘Agde (see Labrune et al., 2007 for geographical reference) with Ib south. In both cases *Lumbrineris latreilli* (Audouin & Milne-Edwards, 1833) was the most abundant species, but the absences (north) or presence (south) of *D. arietina* was the main reason for this separation (Table-3). In the Spanish region, *D. arietina* was rare, but *Hilbigneris gracilis* (Ehlers, 1868) and *Monticellina heterochaeta* Laubier, 1961 were numerically dominant.

TCM assemblages in the French region were clearly differentiated from the other two communities both in sedimentological and composition parameters. This community was typically bounded by the 30 and 40 m isobaths. *Lumbrineris*

latreilli and *Sternaspis scutata* (Ranzani, 1817) were the numerically dominant species. In the Spanish region, these assemblages seem closer to the LSM ones and were characteristic of 50 m and deeper stations. *Hilbigneris gracilis* and *M. heterochaeta* were abundant and common species, but other species such as *Prionospio fallax* Söderström, 1920 and *S. scutata* also reached high densities (Table 3). Off the Costa Brava, the DS assemblage was likely a result of strong currents affecting this area through mechanisms also responsible for the different sedimentary characteristics (Duran et al., 2014). These sediments were characterized by the medium-sized sipunculans (20 mm long average adult size) *Aspidosiphon muelleri* Diesing, 1851 which inhabits empty shells of prosobranchs and *D. arietina*, as well as other small taxa like *Sphaerosyllis taylori* Perkins, 1981 and *Pisione remota* (Southern, 1914) which, due to their average size, surely would have been much more abundant if a smaller mesh size was used.

Potential Good Environmental Status (GEnS)

Five of the eleven descriptors associated with the evaluation of GEnS can directly use data obtained in the REDIT assessment: biodiversity, non-indigenous species, food webs, eutrophication, and seafloor integrity. Our assessment follows these five descriptors. These data also provide regional-scale context within which future studies can evaluate these five descriptors as well as others occurring at different scales (e.g. ecological mechanisms affecting harvests, trophic targets for contamination detection).

Biodiversity - This descriptor has the highest number of potential indicators. The descriptor can be simultaneously assessed at four levels of biophysical organization: ecosystem, landscape, habitat/community, and species states. For the latter two we can directly get indicators for this region from the present study. At the habitat/community level dominant, special, and protected habitats can be identified. One of the dominant habitats in the EUNIS classification (http://eunis.eea.europa.eu/habitats-code-browser.jsp?expand=A#level_A) is Shallow Sublittoral Sediments; the four communities identified in the present work (with their assemblages), LFS, LSM, TCM and DS, represent shallow sublittoral sediments. The areal and geographic extents of these communities are shown in Table 4. At the species level, based on its dominance, five species can be considered characteristic of these communities: *D. arietina* and *O. fusiformis* in shallow sandy environments, and *L. latreilli*, *H. gracilis* and *S. scutata* in muddy environments.

Non-indigenous species - Non-indigenous species, including invasive alien species, have the potential to alter ecosystems (Zenetos et al., 2010) and consequently affect GEnS. The number of such species as well as their range, abundance and impacts on autochthonous communities need to be assessed in the evaluation of this descriptor. Seven polychaete species have been identified as non-indigenous species for the Levantine-Balearic sub-region (Alemany, IEO personal communication). No data are available for the French region. None of the species found in the REDIT campaigns are on this list. The number of new entrants per time unit (i.e. year) is proposed as a numerical indicator for this descriptor. In our case, this number would therefore be 0.

Marine food webs - This descriptor addresses functional aspects of marine food webs, especially the rates of energy transfer within the system, levels of productivity among key components and ecosystem structure in terms of size and abundance of individuals. Although the descriptor is intended to be used for the entire marine food web and addressed from analysis of several trophic levels, estimates of productivity and size at individual levels are needed and may also serve as local proxies. These two indicators are showed in Table 4 for the key characteristic species. The main trophic composition of the three basic communities analyzed can be related to the dominance of the filter feeder *D. arietina* in the LFS community, a much more diverse trophic environment where filter feeders, carnivores and deposit-feeding species are more or less equally distributed in the LSM community, and the biomass dominance of the deposit feeder *S. scutata* in the TCM community.

Eutrophication - Measures of sensitivity to eutrophication can be observed in different ecosystem compartments (e.g. nutrients, chlorophyll, physico-chemical states). Among benthic habitats the relationship between organic enrichment and benthic productivity has been well documented and populations of pioneering species are often used as positive or negative indicators of excessive organic enrichment. The abundance and productivity of *Capitella capitata* (Fabricius, 1780) and closely-related taxa have been used as clear indicators of organic enrichment and eutrophication in the marine environment for many years (see Serrano et al., 2011 for an example of this impact in the studied area). Although *C. capitata* was found in our samples, its average density did not suggest any 'hotspots' of potential enrichment, though sampling density did not provide the spatial resolution required to state that eutrophication on the scale of less than tens of kilometers did not exist in the study area. A second species, normally cited as indicator of organic enrichment and known from the region, *Malacoceros fuliginosus* (Claparède, 1869), did not appear in our samples. It is likely that other species encountered in the present work can be included in the list of indicators, but given the limits of current knowledge, denser sampling along known organic gradients within each biogeographic region is required to identify likely candidates.

Sea floor integrity- The basic indicator of this descriptor gives information on the total area of seabed significantly affected by human activities. Changes in functional diversity and relative abundance of life traits associated with opportunistic and sensitive species can provide estimates of integrity by using different metrics compiled over space and time. The BQI index was used to assess the benthic ecological status of the environment. Table 4 shows the value of this index for the assemblages located in the French part of the study.

Discussion

Among the benthic environments analyzed from the mouth of the Rhône River (France) to Valencia City (Spain), four different polychaete communities with different species and sedimentary characteristics were distinguished, namely the Littoral Fine Sand (LFS), the Littoral Sandy Mud (LSM), the Terrigenous Coastal Mud (TCM), and the Detritic Sand (DS)

Table 4. Indicators used in the assessment of some of the Good Environmental Status descriptors using data for the REDIT mesoscale assessment.

Descriptor 1	**LFS**	**LSM**	**TCM**	**DS**
Habitat extension (ha*103)	200.95	271.30	228.70	14.25

Gulf of Lions (France)

Descriptor 1	**LFS**	**LSM**	**TCM**	**DS**
Species State				
Ditrupa arietina				
Abundance (ind sq m)	616	60	4	
Biomass (mg dw sq m)	962.6	4.6	5.1	
Owenia fusiformis				
Abundance (ind sq m)	233	1	0	
Biomass (mg dw sq m)	106.9	0.1	0	
Lumbrineris latreilli				
Abundance (ind sq m)	21	138	41	
Biomass (mg dw sq m)	18.9	141.0	44.3	
Hilbigneris gracilis				
Abundance (ind sq m)	0	18	4	
Biomass (mg dw sq m)	0	8.5	0	
Sternaspis scutata				
Abundance (ind sq m)	0	2	25	
Biomass (mg dw sq m)	0	23.3	271.9	
Descriptor 2	LFS	LSM	TCM	DS
Non-indigenous species (Nie)				
Number of Nie (#)	0	0	0	
New entrans Nie y-1	0	0	0	
Descriptor 4	LFS	LSM	TCM	DS
Species State				
Ditrupa arietina				
Productivity (mg dw sq m)	853.3	9.2	4.7	
Average biom. (mg dw sq m)	1.56	0.08	1.28	
Owenia fusiformis				
Productivity (mg dw sq m)	131.9	0.2		
Average biom. (mg dw sq m)	0,46	0.10		
Lumbrineris latreilli				
Productivity (mg dw sq m)	19.4	140.2	43.4	
Average biom. (mg dw sq m)	0.90	1.02	1.08	
Hilbigneris gracilis				
Productivity (mg dw sq m)		10.4	0.7	
Average biom. (mg dw sq m)		0.47	0.10	
Sternaspis scutata				
Productivity (mg dw sq m)		12.0	142.7	
Average biom. (mg dw sq m)		11.65	10.88	
Descriptor 5	LFS	LSM	TCM	DS
Species State				
Capitella spp.				
Abundance (ind sq m)	0	0	0	
Descriptor 6	LFS	LSM	TCM	
BQI index	11.70	17.07	19.84	

Northern Mediterranean Spanish coast (Spain)

Descriptor 1	**LFS**	**LSM**	**TCM**	**DS**
Species State				
Ditrupa arietina				
Abundance (ind sq m)	151	15	1	43
Biomass (mg dw sq m)	351.7	44.4	1.4	113.0
Owenia fusiformis				
Abundance (ind sq m)	69	2	1	8
Biomass (mg dw sq m)	125.5	1.2	0.5	2.2
Lumbrineris latreilli				
Abundance (ind sq m)	12	24	10	9
Biomass (mg dw sq m)	5.3	15.0	4.2	9.0
Hilbigneris gracilis				
Abundance (ind sq m)	2	70	87	16
Biomass (mg dw sq m)	0.7	26.1	27.3	0.6
Sternaspis scutata				
Abundance (ind sq m)	0	30	29	0
Biomass (mg dw sq m)	0	59.8	241.5	0
Descriptor 2	LFS	LSM	TCM	DS
Non-indigenous species (Nie)				
Number of Nie (#)	0	0	0	0
New entrans Nie y-1	0	0	0	0
Descriptor 4	LFS	LSM	TCM	DS
Species State				
Ditrupa arietina				
Productivity (mg dw sq m)	279.9	33.1	1.3	87.1
Average biom. (mg dw sq m)	2.33	2.96	1.4	2.63
Owenia fusiformis				
Productivity (mg dw sq m)	106.8	1.4	0.6	3.1
Average biom. (mg dw sq m)	1.82	0.60	0.50	0.28
Lumbrineris latreilli				
Productivity (mg dw sq m)	6.6	17.0	5.3	9.0
Average biom. (mg dw sq m)	0.44	0.63	0.42	1.00
Hilbigneris gracilis				
Productivity (mg dw sq m)	0.9	34.1	37.3	1.5
Average biom. (mg dw sq m)	0.35	0.37	0.31	0.04
Sternaspis scutata				
Productivity (mg dw sq m)		49.6	136.2	
Average biom. (mg dw sq m)		1.99	8.33	
Descriptor 5	LFS	LSM	TCM	DS
Species State				
Capitella spp.				
Abundance (ind sq m)	2	3	0	0
Descriptor 6	LFS	LSM	TCM	
BQI index				

Indicator used in the assessment of some of the Good Environmental Status descriptors using data for the REDIT mesoscale assessment (sq m equals to ind m^{-2}).

communities, following Labrune et al. (2007) classification. Shallow sandy environments of the Northwestern Mediterranean are mostly occupied by the LFS community. Near rocky shores such as the Cap de Creus (Sardá et al., 2012) or highly dynamic deltas such as the Tordera River (Sardá et al., 1999), the LFS community can be replaced by the Littoral Coarse Sand community (LCS). Between shallow sandy and deeper muddy environments, we can find the LSM community, in the past defined as a transition facies (Guille, 1971; Desbruyères et al, 1972–73). This community, normally characterized by sand grains with fine content not higher than 50%, forms a narrow fringe in the Gulf of Lions but is broader and occupies larger areas in the Northern Mediterranean Spanish coast. Where benthic environments are clearly muddy with a high percentage of silt and clay, the species composition is dominated by TCM community. However, as shown in locations off the Costa Brava rocky shores, sometimes oceanographic conditions make sediments change basic profiles and assemblage differences decoupled from bathymetric contours.

Sandy environments at these shallower habitats were easily distinguished by the disproportionate presence of *D. arietina* and *O. fusiformis*. The presence of *D. arietina* was higher in the French region (more than half of the density of the assemblage), and the northern part of the Catalan coast of Spain (one third). Southwards on the Spanish Mediterranean coast the presence of *D. arietina* decreased. Pérès and Picard (1957) pointed out that *D. arietina* was associated with unstable soft sediments and Desbruyères et al., (1972-73) considered this species within the *Nephtys hombergii* Savigny in Lamarck, 1818 community, in which records of *D. arietina* were not so frequent and densities small. Grémare et al., (1998a, 1998b) and Labrune et al. (2007) detected a drastic increase of *D. arietina* populations over recent decades, attributing these high densities to an unidentified response to environmental factors. Sardá et al, (2000) also reported sharp increases of *D. arietina* and *O. fusiformis* after dredging activities on the Catalonian coast. Today, the dominance of the passive filter-feeder *D. arietina* in shallow sandy environments (from 10 to 30 m) in the Gulf of Lions is one of the most obvious components of these benthic habitats. Whether this dominance is related to sediment disturbance, to changes in the sediment release from rivers, to a cascade effect due to other species reductions, or to other unidentified cause or causes, it is worth considering its study and should represent an important aspect of MSFD work. *Ditrupa arietina* was also present in important numbers in the LSM community of the French region.

Owenia fusiformis, *L. latreilli* and *N. hombergii* also deserve comment in these sandy environments. *Owenia fusiformis* populations seem to be more consistent and frequently encountered in this region. Guille (1970) and Desbruyères et al., (1972–73) recorded this species widely in the Northwestern Mediterranean (from well-sorted fine sand in 5 m deep waters to detritic sediments 163 m deep). *Owenia fusiformis* was the second most abundant species on the whole coast in these sediments. Its range covered the entire study area. While *O. fusiformis* was generally restricted to 10 and 20 m stations, *L. latreilli* was the most abundant species at the LSM community in the French region coexisting with populations of another important species *H. gracilis*, in the Spanish region. Desbruyères et al. (1972–73) reported *L. latreilli* as the second most abundant species after *N. hombergii*, however, the presence of the latter species is restricted today and its presence seems to be lower than in past decades. In specific places (e.g. off Barcelona) large alterations to the pattern described in this work have been described and may be a response to organic enrichment (Ros and Cardell, 1992; Cardell et al., 1999; Serrano et al., 2011).

Muddy environments were common at the deepest stations. Nearly all 40 and 50 m stations of the Gulf of Lions and 50 m stations of the Spanish coast were described as mud and grouped in the analysis. In this case, *L. latreilli* in the French region and *H. gracilis* in the Spanish region as well as *S. scutata* can be identified as the most characteristic species following previously identified distributions (Desbruyères et al., 72-73; Galil and Lewinsohn, 1981; Gambi and Giangrande, 1986; Salen-Picard et al., 2003). The exceptions were habitats located off the Costa Brava region where, probably due to stronger currents, mud disappeared and detritic sand environments prevailed.

The MSFD is to be implemented at sub-national economic exclusive zone regional scales. In these regions the essential characteristics and present environmental status of these waters, together with corresponding pressures and impacts, need to be assessed and strategies developed to define GEnS at a regional level. These assessments may also be used at other geographical scales (e.g. administratively defined regions, marine protected zones, tourist destination areas, offshore metropolitan regions). In all these cases, the concept of GEnS in social and ecological assessments can be also applied. Borja et al. (2011) performed an assessment of the environmental status of the Spanish Basque Country following MSFD requirements, and have proposed a method of recombining the eleven descriptors within the MSFD to be applied at a different scale. At whatever assessment scale one works on these issues, the identification, mapping, and consistent evaluation of physical and biological characteristics of benthic habitat types is essential.

The use of the MSFD principles at other scales than the one mentioned in the Directive could be advisable. In any case, the description of GEnS and the interpretation of "good" are key to implementation and relates to human values and worldviews (Mee et al., 2008). Our REDIT work does not pretend to be considered as a kind of standard/reference position for the region in order to set objectives for GEnS, but a "status quo" of its present situation concerning shallow soft-bottom benthic habitats. The definition of "good" for the different descriptors should be determined by those officers managing the region under which the principles of the MSFD would be applied. If GEnS need to be achieved at whatever regional scale an operational definition of GEnS with agreed targets and approaches for integrating assessment results should be approved (Borja et al., 2013).

The mesoscale assessment carried out in the REDIT campaigns contributed to the determination of the distributional

range and extension of the three most widespread communities in the Mediterranean exclusive economic zones of the Gulf of Lions and Northern Mediterranean coast of Spain. Abundance and biomass data for dominant benthic macroinfaunal species are relevant indicators for application of the MSFD descriptor 1, biodiversity and by evaluating productivity and average size, biomass, descriptor 4. The absence of non-indigenous polychaete species within an extensive sampling effort has important implications for descriptor 2, invasive species. Although the Mediterranean is, globally speaking, an oligotrophic sea, metropolitan areas and human activities can result in localised eutrophication. This was the case off Barcelona where several studies (Ros and Cardell, 1992; Cardell et al., 1999; Serrano et al., 2011) illustrated an instance of enrichment and eutrophication; however, eutrophication is not a regional problem based on the assessment carried out. Finally, applying metrics such as the BQI in the assessment of seafloor integrity resulted in a "moderate" (LFS, LSM) or "good" (TCM) state for this benthic environment in the French case; however, this trend was mostly due to a single species (*D. arietina*), the community dynamics of which requires investigation to determine its mechanisms of proliferation.

The distributional range and key characteristics of the soft-bottom communities in the Gulf of Lions and the Northern Mediterranean Spanish coast allowed us to consider its potential use in the assessment of GEnS for the region. Besides individual data for key characteristic species in the ecosystem, the use of several benthic metrics could be useful to evaluate GEnS in the region.

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A new species of *Chaetopterus* (Annelida, Chaetopteridae) from Hong Kong

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Abstract

Sun, Y. and Qiu, J.-W. 2014. A new species of *Chaetopterus* (Annelida, Chaetopteridae) from Hong Kong. *Memoirs of Museum Victoria* 71: 303–309.

A new species, *Chaetopterus qiani* sp. nov., is described based on 18 specimens collected from a fish farm in Hong Kong. This species is small (body length: 11. 5–35.5 mm), with nine, five and 10–16 chaetigers in regions A, B and C, respectively. It belongs to a small group of epibenthic *Chaetopterus* species with long notopodia in region C. This species can be distinguished from other epibenthic *Chaetopterus* by a combination of the following features: up to 16 light-brownish cutting chaetae in A4, wide neuropodia in A9, large wing-shaped notopodia in B1, 10–16 chaetigers in region C, long club-shaped notopodia and a short conical dorsal cirrus in the dorsal lingule of neuropodia in region C. A key to *Chaetopterus* from the Pacific region is provided.

Keywords taxonomy, polychaete, *Chaetopterus*, new species, Hong Kong

Introduction

Chaetopterus is a genus of tubiculous polychaetes characterised by having three distinct body regions. Their body is highly modified for a unique way of filter-feeding: the first pair of the middle parapodia are extremely long and aliform (wing-shaped) and secrete a mucus bag to trap food particles from the water current; the last three pairs of the middle parapodia are fused to form semicircular fans, whose beating, like moving pistons, creates a current of water through the tube (Brown, 1975). *Chaetopterus* species are frequently used as model organisms in studies of reproduction and early development (Irvine et al., 1999; Petersen et al., 2000; Yang et al., 2004), as well as of bioluminescence (Shimomura, 2006).

Despite the common use of *Chaetopterus* as an experimental model, there has been confusion over the number of valid species in the genus. Fauvel (1927, 1953) synonymised several species of *Chaetopterus* with *C. variopedatus* (Renier, 1804), as he considered the variations in body size, number of anterior segments, and length of parapodia to be intraspecific, representing ontogenetic changes or incomplete regeneration following autotonomy. Hartman (1959) went further and synonymised all 25 nominal *Chaetopterus* species with *C. variopedatus*, suggesting that there are no reliable morphological distinctions among them. This cosmopolitan species concept was supported by the observation of Scheltema (1971) on planktonic samples, which indicated that *Chaetopterus* larvae would be able to widely disperse through the transocean current. However, the cosmopolitan species concept was challenged by Petersen (1984a, 1984b, 1997), who stated that '*C. variopedatus*' represents a species complex containing at least ten species. The concept that *C. variopedatus* is a single cosmopolitan species was also refuted by a phylogenetic analysis based on molecular data (Osborn et al., 2007). Indeed, recent studies of *Chaetopterus* have recognised many more species, and their distribution ranges may well be more limited. From Japan, three new species (*C. izuensis*, *C. japonicus* and *C. pacificus*) have been discovered, and three species (*C. cautus*, *C. takahashii* and *C. longipes*) have been redescribed (Nishi, 2001). From the Galapagos Islands, three new species (*C. aduncus*, *C. charlesdarwinii* and *C. galapagensis*) have been published, and two species (*C. longipes* and *C. macropus*) have been redescribed (Nishi et al., 2009). In addition to the aforementioned benthic or epibenthic species, *Chaetopterus* also has one pelagic species (*C. pugaporcinus*) (Osborn et al., 2007), collected at depths of between 875 and 3000 m in Monterey Bay, California, although it was not certain whether these specimens were larvae or adults. Several morphological traits (e.g. tube shape, infaunal or epifaunal habitat, shape and colour of A4 modified chaetae, and shape of cirri in the lateral lobe of region C notopodia) have

Table 1. Major quantitative morphological parameters in *Chaetopterus qiani* sp. nov.

Catalogue number	Body length (mm)	Length of region (mm) A	B	C	Body width (mm)	No. of chaetigers in C	Length of notopodia in B1 (mm)	No. of modified chaetae in A4	Length of tentacle (mm)	Sex	Remarks
Holotype											
MBM179979	24.3	5.4	11.7	7.2	4	12	4.7	10	3.6	♀	
Paratype											
MBM179980	28.3	6.2	10	12.1	4.3	12	5.1	16	2.4	?	
MBM179981	28.6	6.6	10.4	11.6	4.2	15	4.5	16	2.6	?	
MBM179982	21.1	4.5	8.5	8.1	3.5	11	2.7	10	1.5	?	
MBM179983	24.4	*n.r.*	13.1	11.3	2.7	15	3.5	*n.r.*	*n.r.*	♀	a
MBM179984	23.7	4.5	11.4	7.8	3.6	10	3.8	11	2.5	?	
MBM179985	26.1	6.8	10	9.3	3.6	13	4.6	10	2.3	?	
MBM179986	31.2	6.5	14.3	10.4	3.6	11	5.2	12	2.5	?	
AM W46121	26.1	3.4	13.8	8.9	5.9	11	4.3	13	2.5	?	
AM W46122	17.8	3.6	8.6	5.6	3.5	12	3.4	9	1.9	♀	
AM W46123	33.4	7.1	22	4.3	4.5	10	5.7	14	3	♀	
AM W46124	17.5	4.2	7.5	5.8	3.5	10	4.1	10	2	?	
AM W46125	35.6	6.5	23	6.1	4	6	5.6	13	3.3	?	b
AM W46126	27.6	5	13.2	9.4	3.5	16	4.1	15	*n.r*	?	
AM W46127	11.6	3	5.4	3.2	2.4	10	2	8	2.5	?	
AM W46128	26	4.6	14.6	6.8	4.5	12	4.6	11	2.2	♀	
AM W46129	5.5	6.5	n.r.	n.r.	4.0	*n.r.*	4	13	4	?	c

n.r. = character not recorded due to loss of the anterior or posterior part. ? = individuals whose sex cannot be determined by light microscopy. [a]Incomplete specimen without region A. [b]In this specimen, region C has 6 chaetigers only; the posterior part of region C is missing. [c]Incomplete specimen without region C.

been found to be useful for distinguishing *Chaetopterus* species (Petersen, 1984a, 1984b, 1997; Nishi et al., 2000, 2009; Nishi, 2001; Osborn et al., 2007).

Along the Chinese coasts, the only recorded species is *C. variopedatus*, which is likely to be *C. cautus*, according to the description by Yang and Sun (1988). Here we describe *Chaetopterus qiani* sp. nov. from Hong Kong and provide a key to the *Chaetopterus* species in the Pacific region.

Materials and methods

Samples were hand-collected from a floating raft in a fish farm at Port Shelter, Hong Kong. They were fixed in 10% formaldehyde and then transferred to 75% ethanol one week later. The morphology of specimens was observed under a stereomicroscope and a compound microscope. Scaled photographs of the whole body and body structures were taken using a Digital Sight DS-SM camera mounted on an Olympus SZX 16 microscope. One paratype was dehydrated with graded concentrations of ethanol, critical point dried using a BAL-TEC CPD 030 Critical Point Dryer, and observed under a LEO 1530 FESEM scanning electron microscope.

Types are deposited in The Marine Biological Science Museum (MBM) of the Chinese Academy of Sciences, Qingdao, China, and in the Australian Museum (AM), Sydney, Australia (table 1). Description was mainly based on the holotype, with supplementary data from the paratypes showing the variations in morphological characters; SEM micrographs were generated to show the details of the chaetae.

Systematics

Genus ***Chaetopterus*** Cuvier, 1830

Chaetopterus qiani sp. nov.

Zoobank LSID. http://zoobank.org/urn:lsid:zoobank.org:act:DB2F51FF-35F1-4676-890C-F4A4B83FCB68

Figures 1A–G, 2A–H

Material examined. 18 specimens. All type specimens were collected from the fish farm in Port Shelter, Hong Kong (22°20'37.15"N 114°16'58.70"E) on 19 Mar 1998. Holotype: MBM179979, 1 complete specimen in tube with eggs in the parapodia of region C. Paratypes: MBM79980–79986, AM W46121–W46129 and AM W46131 (table 1).

Diagnosis. 9 chaetigers in region A; modified chaetae of A4 light brown, 10–12 in number; wide neuropodia in A9; large wing-shaped notopodia in B1; long club-shaped notopodia and short conical dorsal cirrus in the dorsal lobe of neuropodia in region C; uncini with 7–9 teeth on lateral lobe of C1, and 10–13 teeth on ventral lobe of C1.

Description. Holotype complete with tube (fig. 1A–C), total length 24.3 mm: 5.4 mm in region A, 11.7 mm in region B, and 7.2 mm in region C. Widest part of region A 4 mm.

Region A with 9 chaetigers. Prostomium small, with anterior border rounded, entire. Peristomium extended, completely covering prostomium; wide-horseshoe shaped in anterior view. Two grooved palps extending beyond peristomium, length 3.6 mm (fig. 1A). A pair of eyes present, located at the base of palps. Middorsal ciliated groove extending through region A (fig. 1A). Ventral surface with a long, slender ventral shield (plastron) (fig. 1B): length 4.2 mm, width 2 mm. First 8 chaetigers uniramous, with long, triangular notopodia. Notopodia of A6 longest (figs 1D, 2A). Ninth chaetiger biramous, with long notopodium and stubby neuropodial lobe. Each notopodium with 2–3 rows of light-yellow lanceolate chaetae; dorsal chaetae longer and more slender than lateral ones (fig. 2B, D–E). Notopodia of A4 with 10 modified chaetae. Modified chaetae light brown and club-shaped, with knob-like expanded tip, and arranged in 3 or 4 rows with 2–4 chaetae per row (figs 1E, 2B–C). Neuropodia of A9 with a row of uncini; uncini bluntly D-shaped, with 6–7 teeth in a single row (fig. 2F).

Region B with 5 chaetigers. Digestive gland green in fresh material; colour lost in ethanol-preserved specimens. Parapodia biramous. B1 with enormously enlarged, distally tapering, aliform notopodia extending to A1 (fig. 1A). B2 with elongate parapodium modified as large cupule. B3–B5 fused middorsally, forming enlarged fans. All notopodia of region B without chaetae or uncini. Neuropodia of B1 and B2 with upper and lower uncini lobe, B3–B5 with lower uncini lobe only. Uncini in a single row, similar in shape with uncini in region A; with 5–6 teeth in upper and lower lobe of B1 and B2 (fig. 2G), and 9–10 teeth in B3–B5.

Region C with 14 chaetigers. Parapodia all biramous. Notopodia long, club-shaped with slightly swollen tip (fig. 1A, B). Neuropodia bilobed; lateral lobe with papillary cirrus on lateral side only; ventral neuropodial lobe without cirrus (fig. 1F). Eggs present in neuropodia of holotype (fig. 1G). Uncini of region C similar to those of region A in shape, with 6–7 teeth in lateral neuropodial lobe of C1, and 10–13 teeth in ventral neuropodial lobe of C1 (fig. 2H). Other uncini of region C with 7–9 teeth.

Variation. Several morphological parameters show variations among the type specimens (table 1). The body length varies from 11.6–35.6 mm and the width from 2.4–4.5 mm. The number of modified chaetae in A4 ranges from 8–16. The first notopodia in region B extends to chaetiger A1 in 7 specimens, to A2 in 3 specimens and to A4 in 5 specimens. The number of chaetigers in region C varies from 10–16. Of the type specimens, 5 are females with observable eggs under the body wall, but sex is indeterminable in other type specimens.

Type location and distribution. Currently only known from Port Shelter, Hong Kong.

Etymology. This species is named in honour of Professor Pei-Yuan Qian to recognise his support for polychaete research.

Discussion

Petersen (1984a, 1984b, 1997) separated *Chaetopterus* into two groups according to habitat and tube characteristics: large benthic species with a U-shaped tube, and small epibenthic species with an irregularly shaped tube. According to this classification, *C. qiani* sp. nov. belongs to the epibenthic group, which also includes six other species of *Chaetopterus* from the Pacific region: *C. aduncus* Nishi, Hickman and Bailey-Brock, 2009; *C. charlesdarwinii* Nishi, Hickman and Bailey-Brock, 2009; *C. gregarius* Nishi, Arai and Sasanuma, 2001; *C. izuensis* Nishi, 2001; *C. japonicus* Nishi, 2001; and *C. longipes* Crossland, 1904. This group is characterised by small size (<30 mm in body length) and only a small number of chaetigers in region C (<20). Nishi et al. (2000) proposed 30 characters for distinguishing *Chaetopterus* species. Among them, ten characteristics were used for distinguishing the Pacific species: presence/absence of eyes, morphology of prostomium and peristomium, number of chaetigers in region A, colour and shape of modified chaetae in A4, presence/absence of neuropodia on the last chaetiger in region A, relative size of notopodia in region A, shape and size of notopodia in B1, size and number of teeth in uncini in regions B and C, presence/absence of rudimentary cirri on the lateral lobe of neuropodia in region C, and tube shape and composition.

Based on these morphological characters, *C. qiani* sp. nov. can be distinguished from other species in the epibenthic group of the Pacific region by a combination of characters. It has nine chaetigers in region A, whereas *C. aduncus* has 10–11 chaetigers in region A. The new species has neuropodia in A9, whereas *C. longipes* does not have neuropodia in any of the region A segments. It has eyes and the tubes are muddy, whereas *C. izuensis* does not have eyes and its tubes are sandy. *Chaetopterus qiani* sp. nov. can be distinguished from *C. charlesdarwinii* and *C. gregarious* by the colour and arrangement of the modified chaetae in A4. The A4 modified chaetae of *C. qiani* sp. nov. are light brown and arranged in three or four rows with two to four chaetae per row, whereas the modified chaetae of *C. charlesdarwinii* and *C. gregarious* are dark brown and arranged in one row only. *Chaetopterus qiani* sp. nov. is similar to *C. japonicus* (recorded from the southern Pacific side of central Japan) in the presence of eyes and light-brown modified chaetae in A4. However, the tube of *C. qiani* sp. nov. is irregularly curved or J-shaped and muddy, whereas the tube of *C. japonicus* is U-shaped and has sand and shell fragments on the surface. Besides, *C. qiani* sp. nov. has more chaetigers in region C than *C. japonicus* (12 vs. 6).

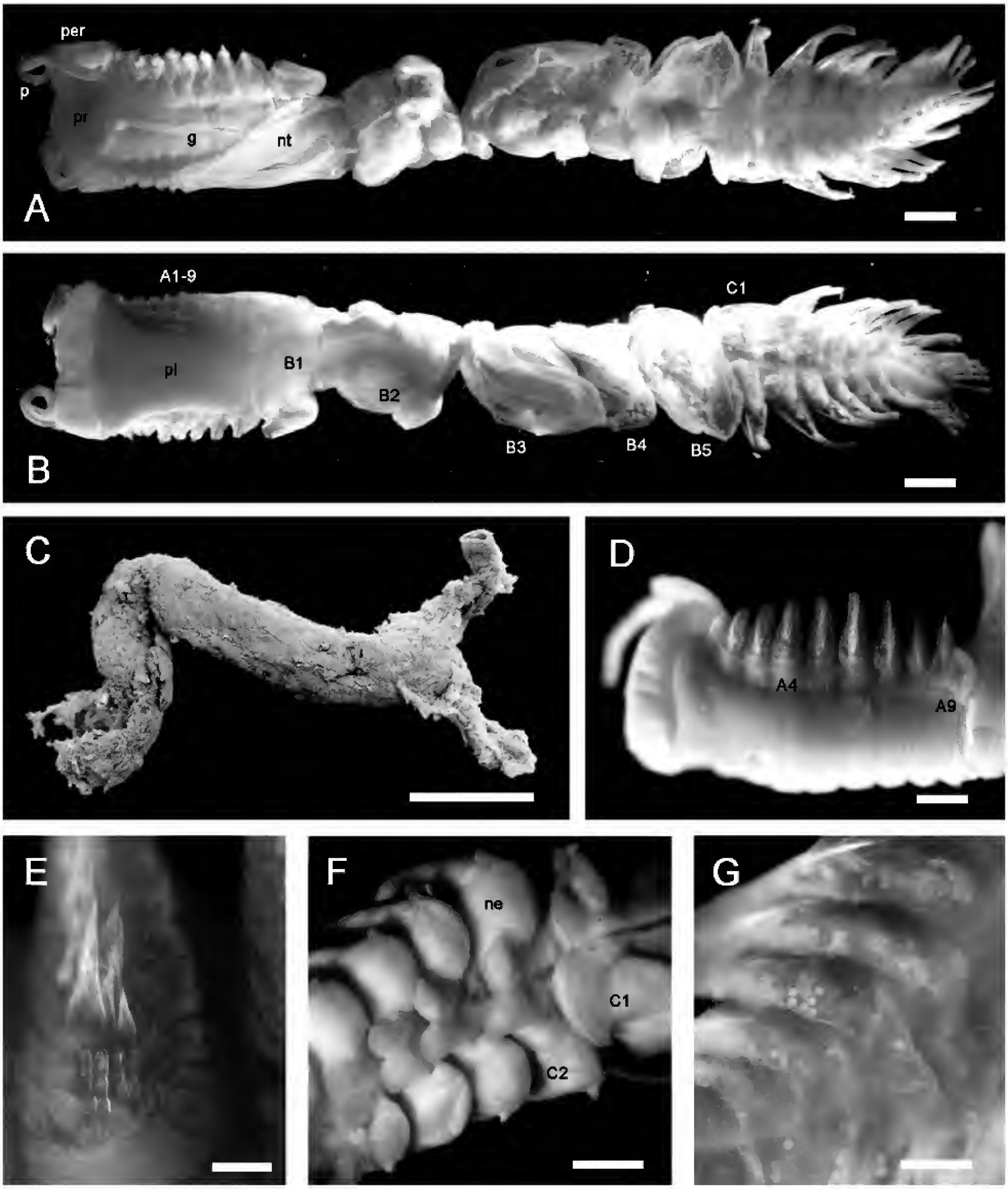

Figure 1. *Chaetopterus qiani* sp. nov., A–C, G: holotype MBM179979. D–F: paratype BU01. A, dorsal view of the whole worm; B, ventral view of the whole worm; C, tube; D, region A, lateral view; E, notopodium of A4 showing modified chaetae; F, ventral view of neuropodia in region C; G, notopodia of region C, showing eggs. A, B and C# = region A, B and C chaetigers, g = mid-dorsal ciliated groove, nt = notopodia, ne = neuropodia, p = palp, per = peristomium, pl = ventral shield (plastron), pr = prostomium. Bar scales: A–B, D, F–G: 1 mm, C: 1 cm, E: 200 μm.

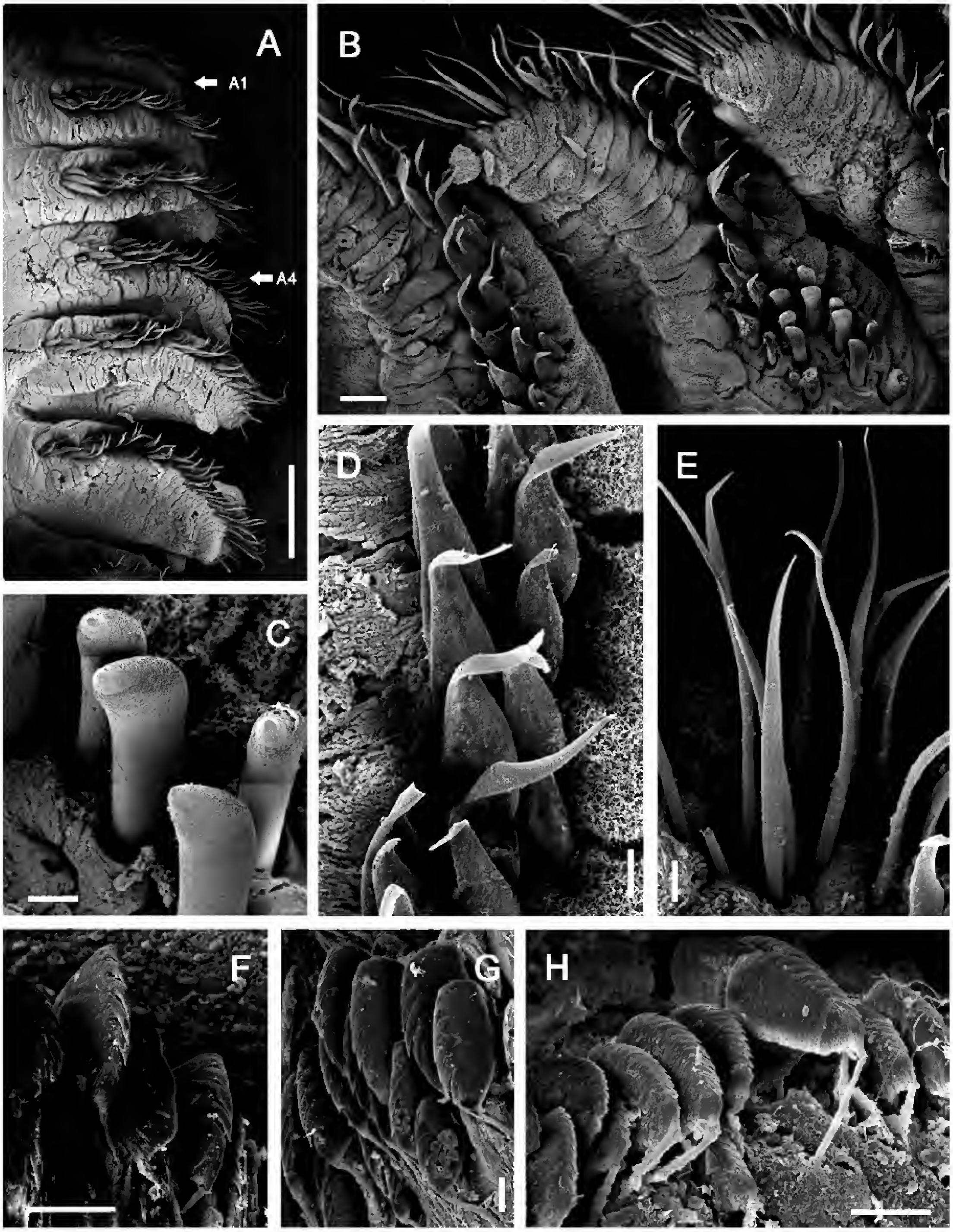

Figure 2. *Chaetopterus qiani* sp. nov., paratype AM W46131. A, A1–6 showing the relative size and arrangement of chaetae, with arrows indicating the positions of chaetigers A1 and A4; B, A3–4, showing the different shapes of the lateral chaetae; C, modified chaetae of A4; D, lanceolate chaetae on lateral side of notopodium; E, lanceolate chaetae on dorsal side of notopodium; F, uncini in neuropodium of A9; G, uncini in neuropodia of B1; H, uncini in neuropodia of C1. Bar scales: A: 500 μm, B: 100 μm, C–E: 20 μm, F–H: 10 μm.

Among the characters that have been used to compare species recorded from the Pacific region (Nishi et al., 2009), some (body width, ratio of length/width of ventral shield, and number of teeth of uncini in each region) exhibit overlap in ranges, but others (shape and composition of tubes, the presence/absence of eye spots, number of chaetigers in region A and region C, number and shape of pairs of A4 modified chaetae, and shape of neuropodial cirri) can be applied to distinguish *Chaetopterus* species. Based on these morphological characters, a key to the *Chaetopterus* spp. is provided.

Key to Pacific species of *Chaetopterus*

1 Benthic, with most of the tube buried in bottom **7**

– Epibenthic, with the tube attached to a solid surface **2**

2 Region A with 10–11 chaetigers .... ***C. aduncus***

– Region A with 9 chaetigers **3**

3 Last chaetiger of region A unilobed .... ***C. longipes***

– Last chaetiger of region A bilobed **4**

4 Tube fragile, made of sand and shell debris; notopodia of B1 straight and slender .... ***C. izuensis***

– Tube parchment-like, made of mud; notopodia of B1 triangular **5**

5 A4 modified chaetae light brown; notopodia of region C club-shaped with slightly swollen end .... ***C. qiani*** **sp. nov.**

– A4 modified chaetae dark brown; notopodia of region C lanceolate with tapered end **6**

6 Region A with a prominent bulbous swelling on the dorsal side of notopodia; uncini with 8–9 teeth in B1, 9–10 teeth in B3 .... ***C. charlesdarwinii***

– Region A without swelling; uncini with 6–7 teeth in B1, 5–6 teeth in B3 .... ***C. gregarius***

7 Neuropodial dorsal cirri of region C long **8**

– Neuropodial dorsal cirri of region C short or rudimentary **9**

8 Neuropodial ventral lobe in region C with both dorsal and ventral cirri .... ***C. cautus***

– Neuropodial ventral lobe in region C with dorsal cirrus only .... ***C. pacificus***

9 Region A with 13–15 chaetigers .... ***C. galapagensis***

– Region A with less than 12 chaetigers **10**

10 Region C with 5–8 chaetigers .... ***C. japonicus***

– Region C with more than 10 chaetigers **11**

11 Region A with a prominent bulbous swelling on the dorsal side of notopodia .... ***C. macropus***

– Dorsal swelling in region A absent .... ***C. variopedatus***

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Chrysopetalidae (Annelida: Phyllodocida) from the Senghor Seamount, north-east Atlantic: taxa with deep-sea affinities and morphological adaptations

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Abstract

Watson, C., Chivers, A.J., Narayanaswamy, B.E., Lamont, P. and Turnewitsch, R. 2014. Chrysopetalidae (Annelida: Phyllodocida) from the Senghor Seamount, north-east Atlantic: taxa with deep-sea affinities and morphological adaptations. *Memoirs of Museum Victoria* 71: 311–325.

Senghor Seamount is located in the north-east (NE) Atlantic Ocean, 550 km west of Senegal, Africa, in the Cape Verde Archipelago. Macrofaunal sampling was undertaken from the summit (~100 m depth) to the base of the seamount (~3300 m depth) during the RV *Meteor* cruise (November 2009). The Chrysopetalidae fauna represents the first record for the family from a tall seamount habitat and is composed of East Atlantic continental margin and deep-sea species. *Dysponetus* sp. 1 is present at the summit and *Dysponetus caecus* at base depths. *Thrausmatos* is recorded for the first time in the Atlantic Ocean, as *Thrausmatos senghorensis* sp. nov., and is found at mid-slope depths only. The taxon with the largest number of individuals, *Arichlidon reyssi*, is most evident at the summit, with one record mid-slope. All Senghor species belong to the only three chrysopetalid genera that possess epitokous, swimming neurochaetae. Adults of *A. reyssi* from the Senghor Seamount and planktonic metatrochophore larvae from the NE Atlantic coast are compared and described in detail. The West Atlantic benthic nectochaete larvae of *Arichlidon gathofi* are also described in the interest of recognising and separating the two cryptic Atlantic *Arichlidon* species.

Keywords North-east Atlantic, polychaete, Chrysopetalidae, swimming neurochaeta, depth distribution, chrysopetalid larva

Introduction

Seamounts are undersea mountains with heights above 1000 m and usually of volcanic origin. They are highly abundant in the Pacific Ocean but occur also in the Atlantic and Indian Oceans (Consalvey et al., 2010). Less than 0.3% of seamounts have been biologically sampled in any detail, and infaunal studies including quantitative sampling methodologies have been scarce (Schlacher et al., 2010; Ramirez-Llodra et al., 2010).

Recent mid NE Atlantic seamount studies include descriptions of the structure and function of seamount ecosystems in the Cape Verde region (Christiansen et al., 2010) and quantitative research into polychaete diversity of the Senghor Seamount (Chivers et al., 2013). Polychaetes are the most common infaunal organisms on NE Atlantic seamounts, with the majority represented by Onuphidae, Syllidae, Eunicidae and Amphinomidae collected by large-aperture-mesh trawl and dredge (Surugiu et al., 2008). Dominant taxa present among the Senghor Seamount fauna are Syllidae, Spionidae, Cirratulidae and Chrysopetalidae collected by quantitative cores (Chivers et al., 2013).

Chrysopetalidae have been recorded from all oceans and are one of the most common polychaetes living in crevicular habitats in tropical, shallow coral reefs of the Indo-Pacific and Atlantic (Watson, 2010). Chrysopetalids are small, often fragmentable polychaetes with golden or silver notochaetal palaeal or spinous fans that cover the dorsum. Separate sexes have been described, and they possess an eversible proboscis with a pair of grooved stylets and an omnivorous, scavenging lifestyle. Over the past 20 years new chrysopetalid taxa have been collected from continental shelves and abyssal oceanic depths associated with wood and whale falls, nodule fields, hydrothermal vents and cold seeps (e.g. Watson, 2001; Dahlgren, 2004).

Specialised swimming neurochaetae have been recorded in species of three chrysopetalid taxa—*Arichlidon, Dysponetus* and *Thrausmatos* (Aguirrezabalaga et al., 1999; Watson Russell, 1998, 2000; Watson, 2001). These three genera constitute the only taxa collected at Senghor Seamount, and swimming neurochaetae are described for the first time in *Dysponetus caecus* (Langerhans, 1880).

Dysponetus caecus and *Arichlidon reyssi* (Katzmann et al., 1974) have been reported over a wide range of depths in the western Atlantic (Watson Russell, 1998; Bőggemann, 2009) and *A. reyssi* in this study from Senghor Seamount. Whether these taxa are able to move between different depths, or whether each taxon comprises a number of cryptic species living at different depths, is discussed.

Differences in larval dispersal mode have been considered one of the main factors related to species genetic connectivity between seamounts, and between seamounts, their adjacent islands and continental margins (e.g. Samadi et al., 2006, Cho and Shank, 2010). Planktonic larvae are typically present in a number of chrysopetalid taxa (Cazaux, 1968; Watson Russell, 1987) and also comprise a major part of the first polychaete fauna settling on artificial reefs, in both temperate and tropical studies (Hutchings and Murray, 1982; Cole et al., 2007).

Planktonic larvae of *Arichlidon reyssi* from the NE Atlantic are described in detail, as are larvae of *Arichlidon gathofi* Watson Russell, 2000 from the West Atlantic, in order to morphologically distinguish the larvae of these two cryptic species. Six- to seven-segmented larvae can be identified to species by examination of palaeal chaetal types of the posterior -most setigers. Clarification is provided of the morphological changes of the first three anterior segments in planktonic metatrochophore larvae and late nectochaetae larvae during metamorphosis and benthic settlement.

Materials and methods

Sampling region. The Senghor Seamount is situated in a meso- to oligotrophic region of the NE Atlantic Ocean and forms an isolated topographical feature located in the Cape Verde Archipelago, ~550 km from the West African mainland at 17.17°N 21.92°W (fig. 1a). The seamount is almost symmetrical in shape, with a summit plateau in ~100 m water depth and a northern base located at a depth of ~3300 m (fig. 1b).

The Senghor summit and upper slopes are composed of craggy areas of bare volcanic rock alternating with patches of coarse sand consisting of coral and bryzoan fragments, sponge spicules, shell gravel from molluscs and barnacles, and some detrital matter. Mid-slope sediments are finer sand covered with shell fragments, and deep-sea stations at the base of the seamount comprise fine, clay-like deposits. Seafloor video footage shows very diverse habitats and faunal communities, especially at the summit, where the seafloor is covered in sediment showing ripple marks (indicating strong currents). Rocks protruding through sediment are overgrown with soft corals, gorgonians and sponges. Deeper stations, at ~800 m depth, have more sparsely populated soft-bottom habitats, but also rocky areas with soft corals and diverse fish communities (Christiansen et al., 2010).

Sampling methods. Chrysopetalid data presented in this study were derived from Senghor Seamount samples collected from a northern transect with four stations (fig. 1B) and an eastern transect with four stations, at depths of ~100–3300 m. No chrysopetalids were found on the southern or western transects (where only two stations were sampled) or at a reference station situated 110 km north of Senghor.

The macrofauna was sampled using a German Multicorer (MUC) with a core diameter of 94 mm, equivalent to 69.4 cm^2 surface area per core. Three deployments were made at each station, with a minimum of three cores taken from each deployment (i.e. a total of nine cores per station). The upper 5 cm of sediment was sliced for faunal analysis, and each sediment sample was placed into a 4% formaldehyde solution for a minimum of two days to fix the tissues prior to sediment washing (to reduce damage to the individuals). The samples were then gently washed on a 250-μm-mesh sieve with filtered seawater (20-μm mesh size) and further rinsed in fresh water before being transferred to 70% ethanol with 2% glycol added.

The macrofauna was initially sorted into major taxonomic groups and counted. The polychaete fauna was then pooled, a wet weight biomass value was obtained, and then sorting (nominally to putative species level) was carried out. The Scottish Association for Marine Science (SAMS) and the German Centre for Marine Biodiversity Research (DZMB) undertook collections at Senghor Seamount, and the chrysopetalid material is housed at the National Museums of Scotland, Edinburgh (NMS), Senckenberg Museum, Frankfurt (SMF) and the Museum and Art Gallery of the Northern Territory, Darwin (NTM). *Arichlidon gathofi* specimens are in the National Museum of Natural History, Washington DC (USNM).

Chaetal terminology follows that of Watson Russell (1991) with designations of notochaetal paleae based on position: i.e. lateral group inserts below the acicula; main group above the acicula; median group at the mid-dorsal line. Abbreviations used: prefix *l* denotes larval; *a* = adult; *p* = primary; *s* = segment. Chaetae: *tc* = transitory chaetae; *la* = lateral paleae; *ma* = main paleae; *me* = median paleae; *en* = epitokous neurochaetae; *sn* = superior group neurochaetae; *if* = inferior group neurochaetae. Anterior end: Roman numerals (I–III) indicate segment number; *dc* = dorsal cirrus; *vc* = ventral cirrus; *dtc* = dorsal tentacular cirrus; *vtc* = ventral tentacular cirrus.

Systematics

Family **Chrysopetalidae** Ehlers, 1864

Thrausmatos Watson, 2001

Thrausmatos dieteri Watson, 2001: 57–66, Figs 1–5 [type species]

Thrausmatos senghorensis sp. nov. Watson, 2014

Zoobank LSID. http://zoobank.org/urn:lsid:zoobank.org:act:B2A32582-0DCC-43C5-840C-6DBC9B1935D4

Figures 2A–D.

Material examined. Holotype: NE Atlantic, Cape Verde Archipelago, Senghor Seamount, East transect, 17°09.66'N 21°53.12'W, some dead coral, 1656.5 m, Core #01, coll. DZMB, Oct 2009, SMF 22963.

Paratypes: same details as holotype, Core 517 #08, coll. SAMS, 1, NMS.Z.2013.160.01; 1, NTM W25388.

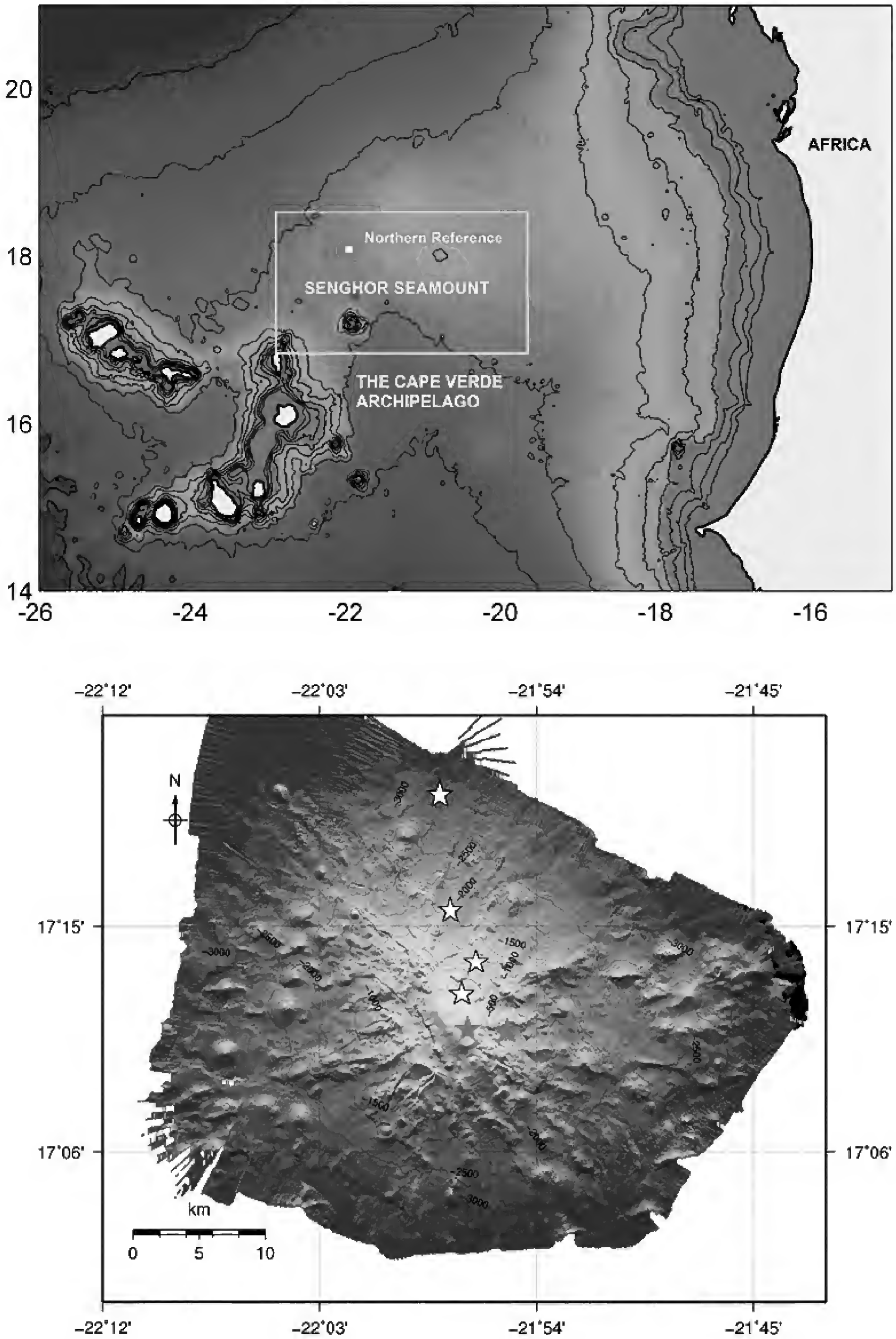

Figure 1. A, Map of Senghor Seamount, located in the Cape Verde Archipelago, NE Atlantic. Data extracted from Smith and Sandwell (1997); dataset created by A. Dale (SAMS). B, Senghor Seamount with the location of transects. Data and map created by Thor Hansteen and Alexander Schmidt, GEOMAR. (A, B, reproduced from Chivers et al., 2013.)

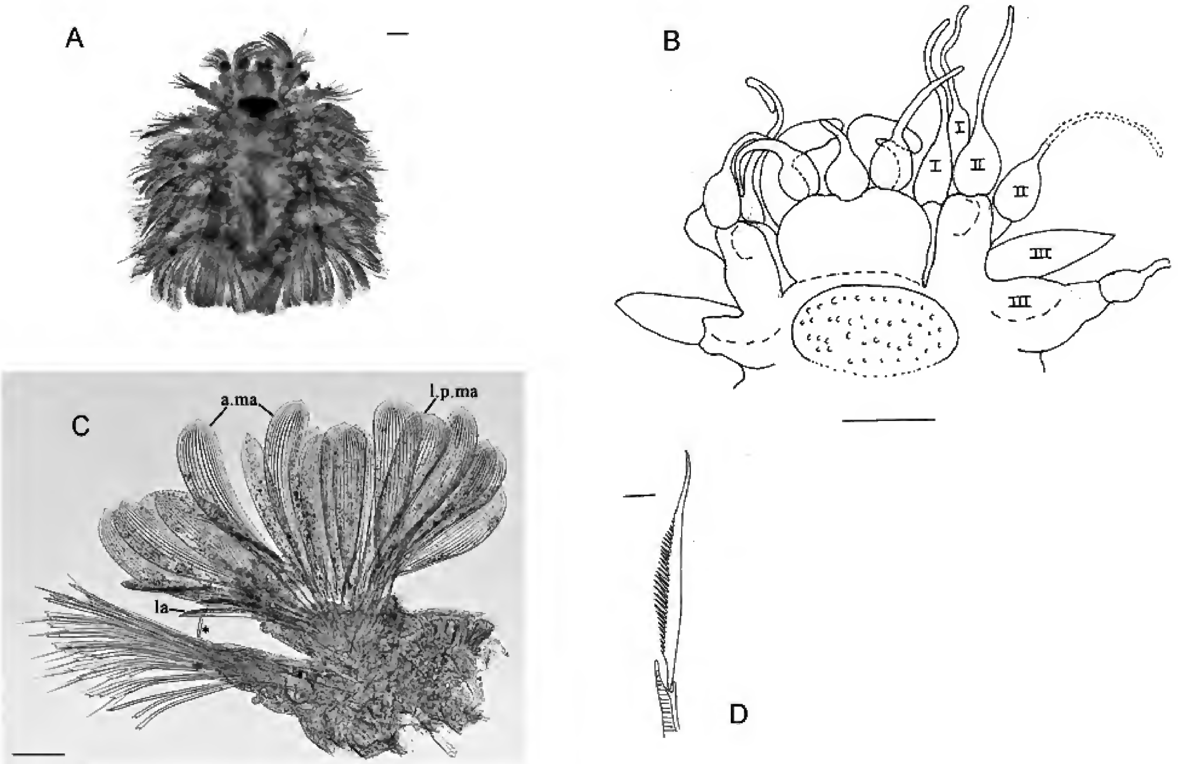

Figure 2. A–D: *Thrausmatos senghorensis* sp. nov., Senghor Seamount, SMF 22963. A, Anterior end, dorsal view, slide preparation; B, anterior end, dorsal view; C, mid-body parapodium, slide preparation; D, detail of superior-most neurochaeta (asterisked in fig. 2C). Scalebars: A-C, 100 μm; D, 10μm.

Description. Based on holotype, an anterior end of 15 segments, length 2.5 mm, width 1.35 mm. Prostomium with subulate median and two lateral antennae; two palps with ovoid bases, subulate distal halves with broad, rounded tips; ovoid caruncle; eye pigment absent. Segment I achaetose with 2 pairs of long cirri; segment II with 2 pairs of long cirri, notochaetal fascicle; segment III biramous with dorsal and ventral cirri, noto- and neurochaetae. Prostomium, caruncle, all ceratophores darker coloured, appear glandular; body epidermis dense with small, rounded structures, probably bacteria. Elongate pharynx with pharyngeal papillae and posterior muscular bulb, extends to segments 8–9 (figs. 2A, B).

Pale golden palaeal notochaetae insert in fans that cover the dorsum. Mid-body notochaetal fascicle with 2–3 short, pointed lateral palae with 5–6 ribs. Main palaea number 10–12 with 16–17 ribs and a couple of lightly raised ribs; medial main a little shorter with same number of ribs; widely spaced horizontal striae. Larval-type main palaea distally with broad 'shoulders'; adult-type main palaea more slender with rounded 'shoulders'; apices prominent (fig. 2C). Very thin, short dorsal acicula; slender dorsal cirri shorter or same length as fan. Neurochaetae number about 30; with long blades and bifid tips. Specialised superiormost fascicle with 2 short falcigers with large basal serrations, inserts supra to overlying long, robust ventral acicula (see asterisk indicating position, fig. 2C; detail, fig. 2D). Mid-superior, middle and inferior neurochaetal groups with very finely serrate falcigerous blades with tiny bifid tips; long, slender ventral cirri (fig. 2C).

Remarks. Elaboration of body shape and posterior end is not possible as all type material is fragmented. The two paratype specimens are both composed of anterior ends of seven segments and display no deviations in chaetal morphology from the holotype.

Thrausmatos species are deep-sea dwellers found only at depths >1000 m. *Thrausmatos dieteri* Watson, 2001 was originally described from hydrothermal vents and seeps from Fiji and New Guinea, SW Pacific. *Thrausmatos* is a new record for the Atlantic and *T. senghorensis* sp. nov. is the first record from a nominal non-chemosynthetic habitat.

Thrausmatos senghorensis individuals are smaller bodied than those of *T. dieteri* and differ in: the more rounded shape of the main palaea and their lack of numerous heavy raised ribs; lesser number of lateral palaea (3 vs. 5–6); shorter dorsal cirri; short falcigers rather than long spinigers of the specialised neurochaetal superior fascicle (fig. 2D); and

absence of pronounced ventral pads. It is very difficult to discern gametes with the opacity of the thick epidermis, which is covered in multiple rounded structures resembling bacteria (fig. 2C). This was also observed in *T. dieteri* (Watson, 2001).

Thrausmatos senghorensis is found at the Senghor Seamount in depths of between 1000 and 3000 m, where ferromanganese crusts are formed at the interface of waters of the oxygen minimum zone and deeper waters (Wang et al., 2011). Although there is no indication of vents or seeps in the area (Chivers, unpublished data), a megacore sample from mid-slope depths on the East transect revealed numerous barnacle plates, suggesting a former vent community that had collapsed (Christiansen et al., 2010). It is possible that the presence of *T. senghorensis* at Senghor Seamount indicates past or as yet undetected hydrothermal activity.

The specialised neurochaetal fascicle appears to be a permanent structure in both small and large individuals of *Thrausmatos* species. These compound chaetae insert in a superior position overlying the ventral acicula of the neurochaetal fascicle. They are much shorter than the transient, long fascicle observed in gametogenic swimming individuals of *Arichlidon* and *Dysponetus*. Larval stages of *Thrausmatos* species are not yet documented.

Distribution and habitat. Thrausmatos senghorensis is found at Senghor Seamount, NE Atlantic, at ~1600 m, among bare volcanic rock and patches of predominantly fine sand and shell fragments.

Etymology. The species name, *senghorensis,* is named after Senghor Seamount.

Dysponetus Levinsen, 1879

Dysponetus pygmaeus Levinsen, 1879: 9, Pl. 1, Figs 1–6 [type species]

Dysponetus caecus (Langerhans, 1880)

Figures 3A, B.

Chrysopetalum caecum Langerhans, 1880: 278–279, NE Atlantic, Madeira Island.—Laubier, 1964: 125–138, Mediterranean, 32 m.

Dysponetus caecus Dahlgren and Pleijel, 1995: 159–173, NE Atlantic, Mediterranean, intertidal to 85 m.—Bőggemann, 2009: 283–296, East Atlantic, Angola Basin, to 5494 m.

Material examined. Dysponetus caecus NE Atlantic, Cape Verde Archipelago, Senghor Seamount, 17°21.82'N 21°57.93'W, North transect, Core 1511 #11, 3241 m, coll. SAMS, Oct 2009, SMF 22964.

Description. Anterior fragment with 13 segments, 3.4 mm long, 1.6 mm wide. Streamlined body, with tapered anterior end. Transparent to silvery notochaetal spines in long fascicles covering dorsum; neurochaetae extend out beyond notochaetae. Prostomium rounded to quadrate, with glandular, ovoid, unpigmented patches on the prostomium, lateral antennae broken, medial papillae (median antenna?) present; 2 ventrolateral palps with broad bases, subulate tips, moderate length. Elongate, single lobe present on posterior margin of mouth; elongate pharynx to segment 7–9 with pair of slender, red-brown stylets (fig. 3A).

Anterior segment I very contracted, with 2 pairs of cirri, dorsal tentacular cirri broken, ventral tentacular cirri present. Segment II biramous with notochaetae and dorsal cirri, neurochaetae, no ventral cirri; notopodia of segment III with notochaetae and dorsal cirri, neuropodia with subulate ventral cirri.

Notochaetal spines long, especially mid-body; with 2 rows of long spinelets. Notopodia with elongate dorsal ceratophores; cirrostyles mostly broken. Shorter dorsal cirri on anterior segments become longer after segment 5. Compound neurochaetae with slender shafts with bifid tip at joint and long, slender, finely serrate blades, minute blade tips unidentate to bifid. Very long-shafted, specialised swimming neurochaetae, numbering 4–6 insert in superior-most position (fig. 3B).

Remarks. In the absence of extant type material of *Chrysopetalum caecum* (Langerhans, 1880) from Madeira Island, NE Atlantic, Dahlgren and Pleijel (1995) designated a neotype from southern France, Mediterranean. The authors redescribed the species and placed it within the genus *Dysponetus*. More recently Böggemann (2009) described *Dysponetus caecus* from abyssal depths off Angola, West Africa, South-east (SE) Atlantic.

Dysponetus caecus Senghor Seamount and Madeira Island specimens of Langerhans (1880: Fig. 9C) have moderate length palps. Palps are lost in Böggemann's specimens of abyssal material from Angola (2009: Figs 20A, B). All Mediterranean material described by Laubier (1964: Fig. 1A) and Dahlgren and Pleijel (1995: Fig. 3A) have longer palps. The arrangement of segments of the anterior end, based primarily on Mediterranean material, and agreed on by Laubier (1964) and Dahlgren and Pleijel (1995), are as follows: segment 1 with 2 pairs of cirri; segment 2 uniramous with notochaetae and dorsal and ventral cirri; segment 3 biramous with dorsal and ventral cirri and chaetigerous lobes. Segment 1 of Senghor material agrees with the above but segment 2 is biramous with chaetigerous lobes and dorsal cirri but no ventral cirri. There appears no sign that ventral cirri were broken off from neuropodia 2, although cirri are fragile and easily lost in dysponetids. More entire material would be needed for confirmation.

A marked increase in notochaetal length has not been observed before in *Dysponetus* (CW, pers. obs.). These longer notochaetae appear in *D. caecus* from Senghor Seamount in segments 9–13, the same segments that possess epitokous neurochaetae (fig. 3A). Slender, non-epitokous neurochaetal blades of *D. caecus* appear spinigerous under the light microscope. Only on highest magnification do the tips of neurochaetae appear unidentate or bifid within the same individual (also observed by Dahlgren and Pleijel (1995)). Neuropodia are very slender with a compressed, dense neurochaetal fascicle. Simple neurochaetae, described in *D. caecus* (Dahlgren and Pleijel, 1995), were not discerned.

Epitokous swimming neurochaetae, similar to those described in planktonic adults of *Arichlidon* species (Watson Russell, 1998, 2000), have been observed in *Dysponetus gracilis* Hartman, 1965 from deep waters of the NE Atlantic by Aguirrezabalaga et al. (1999) and in gametogenic

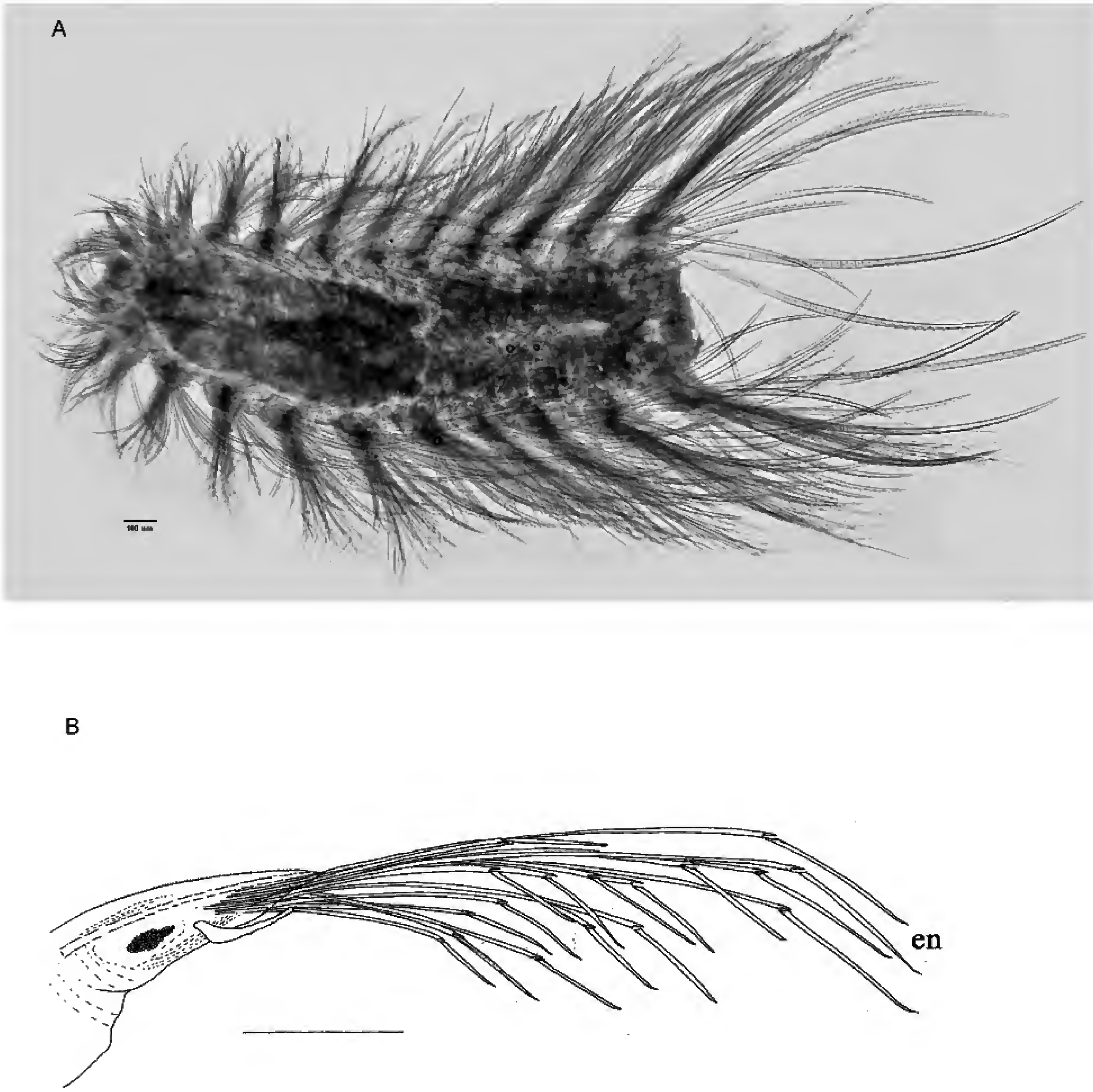

Figure 3. A–B: *Dysponetus caecus,* Senghor Seamount, SMF 22964. A, Anterior end, dorsal view, slide preparation; B, neuropodium XII with superior swimming neurochaetae. Scalebars: A-B, 100μm.

undescribed species of *Dysponetus* and *Pseudodysponetus* (Böggemann, 2009) from southern Australia (CW, unpubl. obs.). These extended, very long-shafted and bladed, compound chaetae insert in a superior position within the neurochaetal fascicle and are recorded for the first time in the male *D. caecus* from Senghor Seamount (fig. 3B).

Very little is known of the larval stages of *Dysponetus* species. The only two instances recorded are of benthic larvae of *Dysponetus pygmaeus* (Watson Russell, 1987) and planktonic larvae of *Dysponetus* cf. *pygmaeus* (Yokouchi, Fig. *in litt.*).

Dysponetus caecus can be separated from its congeners based on a few combinations of characters. However, it is clear that within *D. caecus* there are a number of morphological and ecological disparities between Mediterranean and NE Atlantic forms, e.g. palp length and anterior segment formulae; and large depth differences reported between regions e.g. intertidal

in Mediterranean to ~5000 m off Angola. Morphological revision and genetic analysis of fresh material would help to resolve whether NE Atlantic and Mediterranean *Dysponetus caecus,* as presently understood, is a single species or a complex of cryptic species.

Habitat and distribution. At Senghor Seamount *Dysponetus caecus* occurs among the least-biomass and fine clay-like sediments recorded at the base in ~3000 m depths (Chivers et al., 2013). The nominal distribution of *D. caecus* is currently from 52°N to 19°S in the East Atlantic, including the Mediterranean. *Dysponetus caecus* has been collected from hard and soft substrates, from 1 m to depths of over 5000 m (Dahlgren and Pleijel, 1995; Böggemann, 2009).

Dysponetus sp. 1

Material examined. Senghor Seamount, 17°12.30'N 21°57.70'W, North transect, Core 1509 #01, shelly sand, 133 m, coll. SAMS, Oct 2009, SMF 22963.

Description. One anterior end of 9 segments, 1.2 mm long, 0.9 mm wide. Very small bodied, body fragmented after pharynx level. Prostomium quadrate, with two pairs of large, entire eyes; two small lateral antennae visible on anterior edge of prostomium, median antenna broken, two ventrolateral palps with subulate tips, moderate length. Elongate, single lobe present on posterior margin of mouth; elongate pharynx with pair of slender, red-brown stylets; everted proboscis with ring of small papillae.

Anterior segments: very reduced, achaetose segment I with 2 pairs of long dorsal cirri, ventral cirri bases evident; segment II biramous with notochaetae, long dorsal cirri, neurochaetae, no ventral cirri; notopodia of segment III with notochaetae, dorsal cirri, neuropodia with neurochaetae, small, subulate ventral cirri, not extending past neuropodial tip.

Notochaetal spines moderate length with two rows of spinelets; compound neurochaetae with slender shafts, slender, finely serrate blades, minute blade tips unidentate to bifid.

Remarks. Overall anterior end and chaetal characters agree between the shallow and deep *Dysponetus* individuals, but the smaller *Dysponetus* sp. 1 possesses two pairs of large red eyes, and all *D. caecus* material from both shallow and deep waters have been described in the literature as lacking eyes.

The only dysponetid described with eyes from the NE Atlantic is *Dysponetus joeli* Olivier, Lana, Oliveira & Worsfold, 2012 recorded from the English Channel in a maerl, shallow-water habitat. Without examining original *Dysponetus joeli* material, it is not possible to compare the single Senghor Seamount specimen based on the poorly preserved material figured and described in the literature.

Habitat. Dysponetus sp. 1 is found at 133 m at Senghor Seamount among coarse sediments.

Arichlidon Watson Russell, 1998

Arichlidon hanneloreae Watson Russell, 1998: 160, Figs 1–4 [type species]

***Arichlidon reyssi* (Katzmann, Laubier & Ramos, 1974)**

Figures 4A, B.

Bhawania reyssi Katzmann, Laubier & Ramos, 1974: 313–317, Fig. 1A–G. Type locality: Adriatic Sea.

Paleanotus heteroseta Rullier, 1964: 142–3. Cape Verde Islands.

Chrysopetalum debile Cazaux, 1968: 536–541. Arcachon, France (larvae).

Arichlidon reyssi Watson Russell, 1998: 159–176, Figs 4C, 6G, H. Adriatic, Mediterranean, Cape Verde Islands.

Arichlidon reyssi Watson Russell, 2000: 465–477, Fig. 1A–D. Eastern Mediterranean

Material examined: NE Atlantic, Cape Verde Archipelago, Senghor Seamount, East summit, 17°12.30'N 21°53.12'W, shelly sand, 133.6 m, Core 1510 #08, coll. SAMS, 14, NMS.Z.2013.160.02; 17°10.62'N 21°56.83'W, 103.1 m, coarse sediment, Core 1531 #11, coll. SAMS, 3, NMS.Z.2013.160.03; 17°12.29'N 21°57.69'W, 132.4 m, Core #01, coll. DZMB, 4, NMS.Z.2013.160.04; 17°10.62'N 21°56.84'W, 103.1 m, Core #01, coll. DZMB, 3, NMS.Z.2013.160.05; 17°10.62'N 21°56.82'W, 102.7 m, Core #01, coll. DZMB, 1, NMS.Z.2013.160.06; East summit, 17°12.30'N 21°57.70'W, 133.6 m, shelly sand, Core 1510 # 12, coll. SAMS, 2, NMS.Z.2013.160.07; East summit, 17°12.30'N 21°57.70'W, shelly sand, 133 m, Core1509 #02, coll. SAMS, 2, NMS.Z.2013.160.08; 17°09.66'N 21°53.12'W, dead coral, 1656.5 m, Core 1517 #08, coll. SAMS, 2, NMS.Z.2013.160.09; East summit, 17°12.30'N 21°57.70'W, shelly sand, 133.6 m, Core 1510 #10, coll. SAMS, 3, NMS.Z.2013.160.01; 17°10.62'N 21°56.82'W, Core #04, 102.7 m, coll. DZMB, 2, SMF 22965; 17°1210.62'N 21°56.84'W, Core #05, 103.1 m, coll. DZMB, 6, SMF 22966; 17°12.29'N 21°57.69'W, Core #07, 132.4 m, coll. DZMB, 17, SMF 22967; 17°10.62'N 21°56.84'W, Core #08, 103.1 m, coll. DZMB, 2, SMF 22968; 17°10.62'N 21°56.82'W, Core #10, 102.4 m, coll. DZMB, 8, SMF 22969; 17°12.29'N 21°57.69'W, Core 864 #02, 132.4 m, coll. DZMB, 10, NTM W 025386; East summit, 17°12.30'N 21°57.70'W, Core 1509 #01, shell sand, 133 m, coll. SAMS, 3, NTM W25387.

Description. Largest individual measuring 50 segments, length 5.0 mm and width 1.1 mm. Body relatively short, broad, with silver to pale-golden palaeal fans, often with brownish scale bands, covering dorsum. Prostomium with two pairs of violet-black eyes often fused, forming rectangular block visible beneath palaea of anterior segments (fig. 4A). Segment I with two pairs of dorsal and ventral tentacular cirri; segment II with palaeal notochaetae, dorsal cirri, neurochaetae, ventral cirri absent. Lateral palaea fascicle intergrades smoothly with main palaea fascicle; distinctive group of asymmetrical ornate median palaea interlock middorsal line forming smooth convex ridge. From segment VI median group palaea number 3–5. Long lateral-most median palae appears first at segment VI and continues down body as tallest palae in entire fan (fig. 4B). Dorsal surface of notochaetal palae with tubercules and raised serrate ribs. Neurochaetae comprising superior group of spinigers; mid group with strongly dentate falcigers; inferior group falcigers with short, broad, curved articles with smooth to minutely serrate margin and blunt tip.

Remarks. One character, not reported on previously, and observed in 67 individuals of *Arichlidon reyssi* at Senghor Seamount and in material of all other re-examined *Arichlidon* species, is a distinctive paired structure at a level near the top of the pharynx. It is composed of two small, brownish,

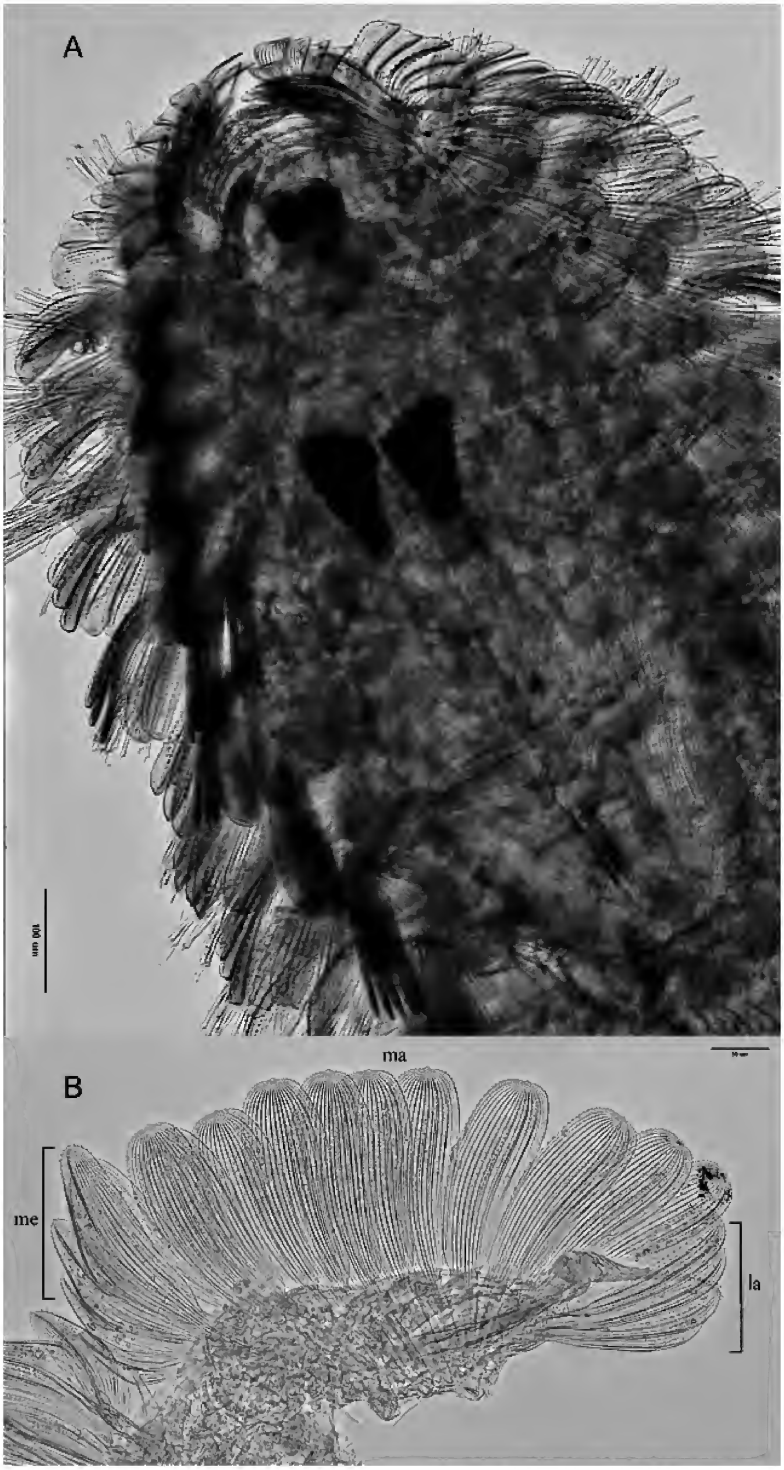

Figure 4. A–B: *Arichlidon reyssi*, adult, Senghor Seamount, NMS.Z.2013.160.09, slide preparations. A, Anterior end; B, mid-body notopodium from anterior end. Scalebars: A, 100 μm; B, 50μm.

triangular structures sitting opposite each other, either side of the pair of stylets (fig. 4A). When dissected they spill out densely packed, tiny, golden-brown globules; function currently unknown. They are reminiscent of the oil globules common to many chrysopetalid species that occur as larger, singular globules inside parapodia (Watson, 2012).

Epitokous swimming neurochaetae, described from both benthic and planktonic adults in all nominal *Arichlidon* species (Watson Russell, 1998, 2000), were not seen in any *Arichlidon reyssi* individuals in the present study.

Adult specimens of *Arichlidon reyssi* from the Cape Verde Archipelago (Maio, Brava and Boavista Islands) in sponge, shell and sediment samples, depth 20–425 m, were included in the description of the new genus *Arichlidon* and a redescription of *A. reyssi* from the Adriatic and Mediterranean Seas and NE Atlantic (Watson Russell, 1998). *Arichlidon reyssi* specimens observed in this present study from Senghor Seamount morphologically agree with the former Cape Verde material examined in all characters of body shape, size, colouration, notochaetal and neurochaetal characters, including numbers of palaeal ribs and chaetal types.

Previously, *Arichlidon reyssi* have been collected in moderately large numbers (e.g. 82 individuals from one station) and over large depth ranges (10–4000 m) in the Eastern Mediterranean (Watson Russell, 1998). At Senghor Seamount, *A. reyssi* ranges from the summit at 102 m to mid-slope depths of over 1000 m. In both cases, no discernible morphological differences were found between individuals at different depths.

Arichlidon is one of a number of chrysopetalid taxa that possess primarily cryptic species with a very conservative morphology. Watson Russell (2000) described a new species, *Arichlidon gathofi* from the western Atlantic, and compared it with *A. reyssi* on the basis of one character in particular. In *A. reyssi*, the long lateral-most median palae, with a higher number of ribs, is taller than the main fan (fig. 4 B); in *A. gathofi*, the lateral-most median palae, with a slightly lesser number of ribs, is the same height or shorter than the main fan (fig. 6E). This singular median-palae is evident in mid-body segments in juvenile and adult material examined and dissected from both species (Watson Russell, 2000: 476). In order to identify chrysopetalid larvae to species, it is essential to study chaetal patterns throughout the entire body. In the interests of distinguishing Atlantic *Arichlidon* larvae to species, and to elaborate on the sequence of changes in the morphology of planktonic to benthic individuals, larvae of *A. reyssi* and *A. gathofi* are described below.

Distribution and habitat. Benthic adults of *Arichlidon reyssi* are found from the Mediterranean, NE Atlantic coast, and the islands and seamount of the Cape Verde Archipelago. Among the Senghor Seamount chrysopetalid fauna, *A. reyssi* comprises the largest number of individuals, which predominantly dwell in coarse sediments at the summit at ~100 m, among the largest polychaete biomass recorded. There is also one record from mid-slope at 1651 m.

Arichlidon reyssi metatrochophore planktonic larvae

Figures 5A–F.

Material examined: NE Atlantic, France, Arcachon, from plankton outside Marine Station, Nov 1987, coll. C. Cazaux, 3 entire specimens all 6 segments.

Description based on planktonic specimens. 1: Length 480 μm, width 440 μm; 2: length 640 μm, width 440 μm; 3: length 720 μm, width 520 μm; NTM W25385.

Broad, ovoid bodies filled with dense oily droplets; conspicuous fascicles of long, brown, latero-anteriorly directed transitory notochaetal spines in first chaetigerous segment (fig. 5A). Smallest larva 1 with notochaetal fans more folded and bare mid dorsal line; larvae 2 and 3 with notochaetal palaeal fans spread over dorsum from segments II–VI; compound falcigerous neurochaetae from segments II–VI. All larvae possess large rounded epispheres with three pairs of eyes; largest pair in anterodorsal position with apparent lenses, smaller pairs more dorsal. Larva 1 prostomium with small, unpaired, anterolateral cirrus (developing lateral antenna?); larva 3 with circular hyaline patch mid-episphere and developing mouth. No median antennae, palps or nuchal organs visible.

Small ciliate 'buds' present each side of body at posterior latero-dorsal edges of episphere at dividing line between head and trunk (nascent adult segment 1). Larval segment I with two pairs of larval tentacular cirri, longer than following cirri; inserting at the same level as the transitory notochaetae. Transitory notochaetae insert in large, rounded dorso-lateral lobe with 2 aciculae; number ~15, with larger spinelets along entire lateral edge and minor spinelets in another plane along part of length (figs 5A–D).

Segment II, ventral view: very small neuropodial rami present and directed towards mid-body ventral line i.e. not laterally; with fascicles of spinigerous neurochaetae, ventral cirri absent. Neuropodia III–V1 with subulate ventral cirri (fig. 5C).

Notopodia of segments II–III with larval primary palaea types only: with 2–4 lateral palaea, 1–3 short spines, 4 large symmetrical main palaea and 2 broad asymmetrical palaea in medial-most position. Notopodium of segment III with 2 lateral palaea with 8–12 ribs, 5–7 main palaea with 17–21 ribs and 4 symmetrical median palaea. Larval main palaea distally rounded. Subsequent notopodia with 1 small spine overlying dorsal aciculum; notopodia of segments II–VI with relatively short, subulate dorsal cirri (fig. 5B).

Segments IV–VI notopodium with adult chaetal types replacing larval types. Notopodia of segment IV with 4 lateral palaea with 6–14 ribs; 4–5 main palaea with 15–19 ribs, including large, slightly asymmetrical subunit 1 palae with 19–20 ribs and 3 raised serrated ribs; 5–6 median palaea grading in size and degree of asymmetry with 7–14 ribs, including tall lateral-most one with 2–3 raised and serrated ribs as tall as or taller than main palaea group) (fig. 5E). Notopodia of segment V with 3 lateral, 4 main and 5 median; notopodia 6 with small notosetal fascicle comprising 1–2 slender lateral, 2 main and 3 short median palae. Adult main palae distally squarer (fig. 5F).

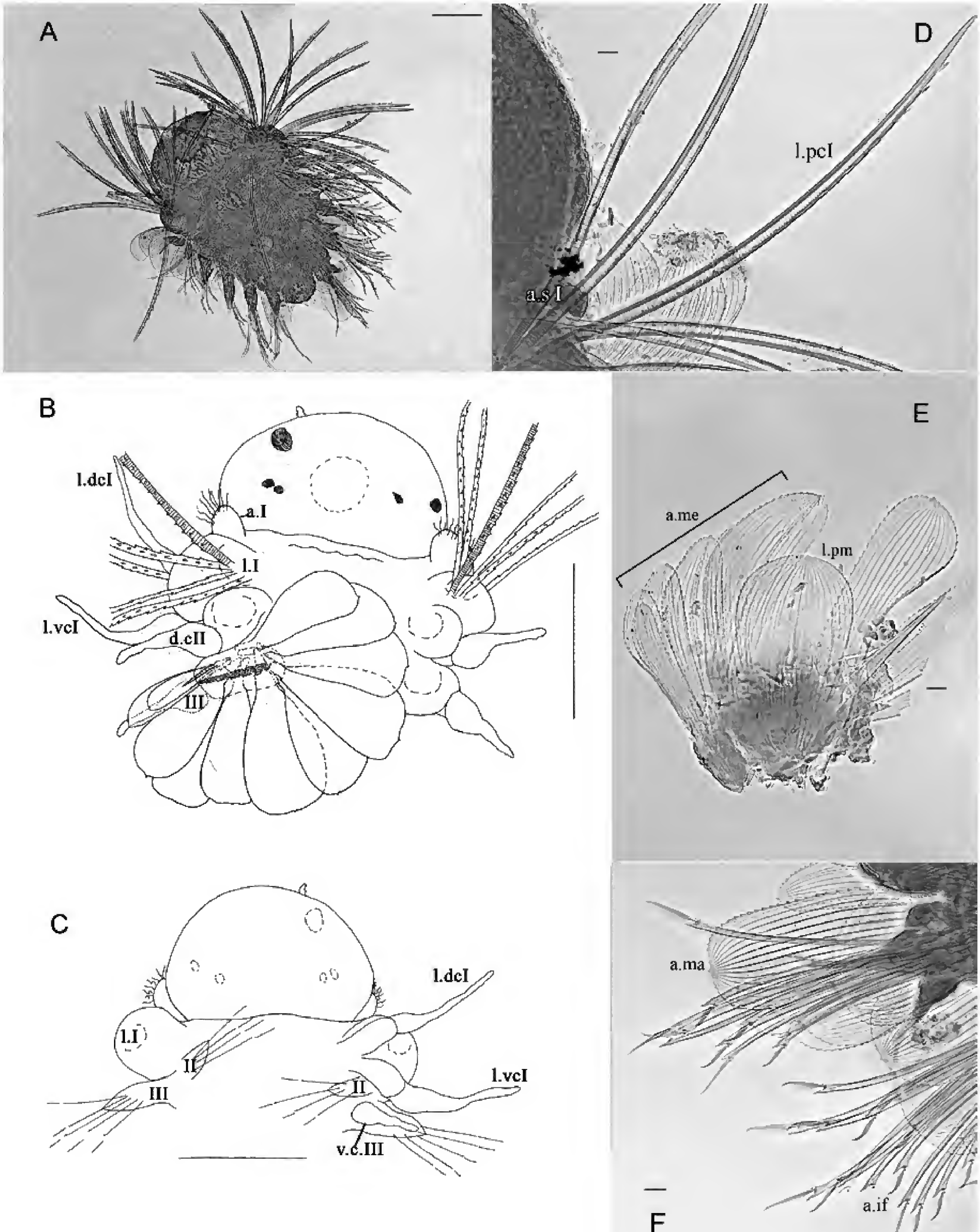

Figure 5. A–F: *Arichlidon reyssi* 6-segmented larva, Arcachon, NE Atlantic, NTM 25385; A, D–F: slide preparations. A, Entire larva, dorsal view; B, anterior end, dorsal, left side detail (transitory chaetae drawn in part); C, anterior end, ventral view, left side detail; D, detail of anterior end of fig. 5A; E, notopodium segment IV; F, neuropodia segments IV and V. Scalebars: A, 50μm; B-C, 100μm; D-F, 10 μm.

Segment III neuropodia mainly with falcigerous neurochaetae with slender, narrow blades; recognisable adult chaetae and adult types from neuropodia of segments IV–VI. Neuropodium of segment IV with 1 superior spiniger, 2–4 mid-superior falcigers with long blades, 4 mid-inferior falcigers with shorter blades and 6–8 inferior falcigers with typical adult smooth, short, curved blades (fig. 5F). Neuropodium of segment VI with 1–2 superior long, narrow-bladed falcigers and 2–3 lower falcigers with shorter, slender blades. All neuropodia with 1 short, simple spine overlying ventral acicula. Pygidium composed of ventral median conical protruberance and dorsal rounded structure with two lateral anal cirri.

Remarks. Cazaux (1968) provided detailed figures of the early development of a species he identified as *Chrysopetalum debile*, collected at different stages from the plankton at Arcachon, NE Atlantic. The '*C. debile*' identification was based on one of a number of chrysopetalid species present in the region, and original material was subsequently lost. Study of recent material of metatrochophore chrysopetalid larvae from the same locality and described in this paper, confirms Cazaux's material as most likely belonging to the species *Arichlidon reyssi*.

Behavioural observations in Cazaux's 1968 paper include a description of the planktonic larvae not feeding but living on their reserves and at the slightest touch rolling into a ball, becoming bristly like a 'Chaetosphera' larvae. He observes there is a planktonic duration of at least three weeks between metatrophore 1 to nectochaete 1, and their presence in stations located between the ocean and inner estuary of the Bay of Arcachon between October and December. Bhaud *in litt.* mentions their presence in the Western Mediterranean between August and October.

Distribution. Planktonic larvae of *Arichlidon reyssi* have been reported from the Mediterranean and NE Atlantic coast.

Arichlidon gathofi benthic nectochaete larvae

Figures 6A–F.

Arichlidon gathofi Watson Russell, 2000: 465–477

Figures 1–5.

Material examined: Paratypes. USA, off North Carolina, western Atlantic, Stn. 2606, 34° 35'N 75° 52'W, 45 m, coll. RV *Albatross*, 18 Oct 1885, USNM 186017. Note: 148 individuals were collected; among these were 36 juveniles and 4 late nectochaete larvae, the latter described herein.

Description based on benthic specimens. 7 segments: length 520 μm, width 500 μm (fig. 6A, B); 7 segments: length 460 μm, width 460 μm; 8 segments: length 540 μm, width 460 μm; 10 segments: length 700 μm, width 500 μm; 11 segments: length 840 μm, width 52 μm; 14 segments: length 920 μm, width 520 μm.

Larvae of 7 segments with broad, ovoid body shape with palaeal fans fully extended over dorsum, neurochaetae extending out beyond palaea; dense oil globules in gut. Rounded prostomium with faint red eye pigment visible; short, stout median antenna inserts on anterior edge of prostomium; lateral antennae, palps and nuchal fold absent. Segments I–III in adult configuration (figs 6A, B). Segment I more visible in ventral view, with two pairs of dorsal and ventral tentacular cirri (fig. 6B).

Notopodium of segment II with 2 narrow palaea, 6–8 ribs. Notopodium of segments II–IV include primary, expanded palaea in medial position with 15–16 ribs (fig. 6C). Segments V–VII with adult type, slimmer, asymmetrical median palaea, numbering 2–3, shorter than main fan, with 11–14 ribs (fig. 6D). Broad, asymmetrical medial-most, subunit 1, main palae (*A. gathofi* species character) present posterior segments VI–VII. Prominent, curved notochaetal spine originating from lateral group (continues into adult); subulate dorsal cirri present on all notopodia (figs 6C, D).

Neurochaetae of segment II all spinigers; neurochaetae of segments III–VII include 2–3 superior spinigers; adult groupings of mid-superior and mid-inferior falcigers; typical short, curved articles of inferior falcigers. Pygidium composed of slender ventral cone and dorsal structure with two filiform anal cirri.

Post-larvae and juveniles 8–14 segments with body slightly tapered anteriorly and posteriorly; neurosetae not extending out beyond palae. Prostomium smaller with two pairs of eyes, longer, subulate median antenna, two lateral antenna and two ventral, long, cylindrical palps. Triangular mouth fold posterior to palps, pair of stylets evident in pharynx; raised glandular nuchal fold present posterior to prostomium. Increasing numbers of adult main palaeal notochaetae and neurochaetae with increasing body segments.

Remarks. Chrysopetalid notochaetal palaea, spines and neurochaetal shafts are composed of internal longitudinal ribs and horizontal diaphragms (Westheide and Watson Russell, 1992). The appearance of the first chaetae arises in the trochophore after initiation of the first larval segment. These long, brown, spinulose provisional chaetae are internally striated. The metatrochophore 4-segmented larvae develop compound falcigerous neurochaetae with striated shafts, and the generation of the sixth segment initiates primary, laterally folded, notochaetal palaeal fans and spines, all striated internally (Cazaux, 1968; Watson Russell, 1987).

This construction of internally striated chaetae creates maximum strength and lightness for larvae and adults found mid-water. Adult chrysopetalids may also possess epitokous, swimming neurochaetae, as first described for *Arichlidon gathofi* collected from the plankton (Watson Russell, 2000, Fig. 5A, and reproduced in this paper as fig. 6F).

Mileikovsky (1962) observed that the long provisional chaetae found in chrysopetalid, sabellariid and some 'Chaetosphaera' spionid trochophore larvae are probable convergent structures suited to a similar pelagic mode of living, with larvae able to be transported very long distances. There is no record of chrysopetalid teleplanic larvae, but chrysopetalid metatrochophore larvae have been collected from vertical plankton tows from the surface down to 100 m, in 3000–4000 m depth in the Gulf Stream, NW Atlantic (Mileikovsky, 1962). Original material was lost but its identity is inferred from his figures as belonging to either the genus *Arichlidon* or the deep-sea-dwelling *Strepternos* (see Watson Russell, 1997).

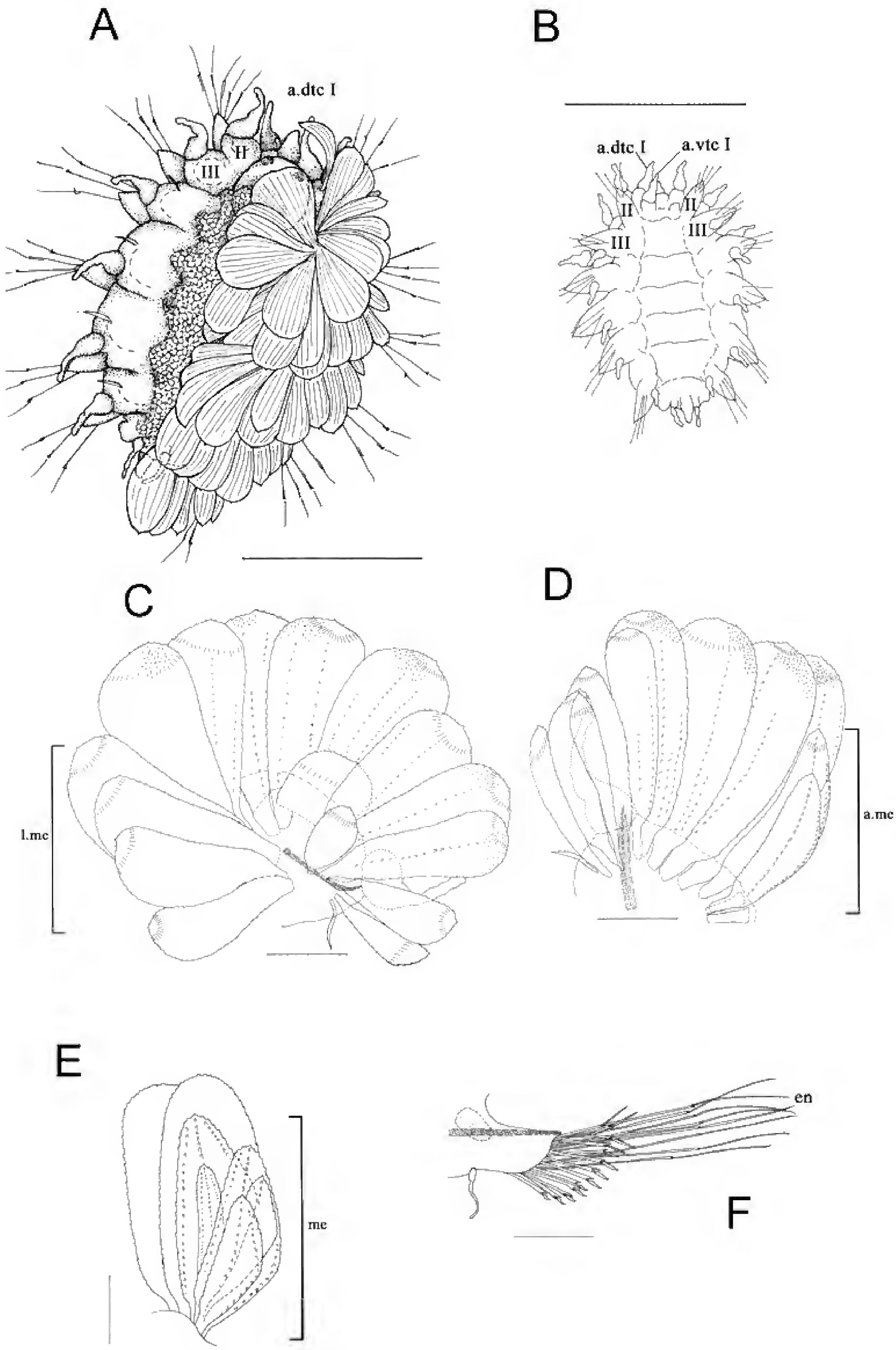

Figure 6. A–F: *Arichlidon gathofi,* 7-segmented larva, Carolina, West Atlantic, USNM 186017. A, Entire larva, dorsal view; B, ventral view of A; C, notopodium segment IV; D, notopodium segment VI (figs A, C, after Watson Russell, 1987: Figs 28.4, 6: as 'new genus 1'). E, *A. gathofi,* adult, mid-body notopodium, detail median fascicle; F, mid-body neuropodium with epitokous swimming neurochaetae (figs E, F after Watson Russell, 2000: Figs 1D, 5A). Scalebars: A, 200μm; B, 350μm; C-E, 40 μm; F, 100μm.

Distribution and habitat. Arichlidon gathofi is found from North Carolina, USA to Panama, Central America, western Atlantic. Benthic habitat varies from silty sands in the Gulf of Mexico to algal, sea-grass, shell and coral rubble substrates of the islands of the north and south Caribbean; 1–106 m.

Remarks on the larval morphology and development of Arichlidon reyssi and A. gathofi. Metamorphosis at the 6–7-segment stage occurs at benthic settlement and includes loss of larval notopodia 1 (comprising larval pair of cirri and transitory, provisional chaetae, figs. 5A–D) and development of adult segment I in a dorsal/ventral plane. The episphere reduces in size as it differentiates into a more adult prostomium and its appendages develop. Concurrent with these changes is development of adult notopodia II and III with forward rotation and part fusion, particularly evident in dorsal view; larval primary palaea are lost on notopodia II and replaced by a few, short adult palaea (fig. 6A). The nuchal fold begins to take shape as a result of these former changes and forms part of the retraction mechanism of the anterior end. A discreet caruncle, as postulated by Cazaux (1968), is found primarily in *Chrysopetalum* and is not present in *Arichlidon* species.

Adult segment I is developed from the ciliate buds seen in the larvae at the conjunction of the episphere and trunk (fig. 5A–D). From a 7-segmented larvae onwards, this segment I appears reduced and fused in part to the prostomium. It supports a pair of dorsal and ventral cirri that are often more visible in ventral view. These later-formed adult cirri are shorter than the larval pair and are approximately the same size as those dorsal cirri seen in segment II (fig. 6A, B). At no developmental stage are chaetae present on adult segment I in *Arichlidon* species, and the term 'tentacular' is therefore retained as a descriptor for the cirri of this segment.

A similar series of morphological changes has been described for the larval deep-sea chrysopetalid *Strepternos didymopyton* Watson Russell, 1991, which has the same anterior end schema, i.e. segment I with two pairs of tentacular cirri, segment II with notopodia, chaetae, neuropodia with chaetae, ventral cirri absent (Watson Russell, 1997). In *Strepternos* and *Arichlidon*, the small neuropodia 1 does not at any time possess ventral cirri (fig. 5C, 6B). It has been the contention of some authors, e.g. Perkins, 1987, that there has been loss of ventral cirri from this segment during ontogeny.

Identification of Atlantic Arichlidon larvae to species. Chaetal patterns in the midposterior body of chrysopetalid larvae can be used for identification to genus and species (Watson Russell, 1987). The shape of the main palaea (and particularly the inferior-most curved, falcigerous neurochaetae from posterior segments) identify the above larvae as belonging to the genus *Arichlidon* (fig. 5F). Adult lateral, main and median palaeal types are present from segments IV–VI, with the overall highest numbers of adult chaetal types present in segments IV–VI in *A. reyssi* and segments V–VII in *A. gathofi.* The tall lateral-most median palae—a distinguishing species character for *A. reyssi*—is clearly visible from segment IV (fig. 5E); the shorter, broader median palae visible from segment V in *A. gathofi* (fig. 6D).

Discussion

Dispersal mode and depth ranges of chrysopetalid species at Senghor Seamount

The polychaete fauna of the Cape Verde Islands represents West African species, American elements absent from the continental African plateau, small numbers of endemic species, and species from the southern limit of the NE Atlantic and Mediterranean (Ruillier, 1964). Senghor Seamount chrysopetalid species present in this study comprise a predominantly eastern Atlantic fauna. *Thrausmatos* species are deep-sea dwellers found only at depths greater than 1000 m, and the new species, *T. senghorensis*, is potentially a NE Atlantic seamount endemic. *Dysponetus caecus* and *Arichlidon reyssi* are regional benthic species: *A. reyssi* from the Mediterranean Sea and the NE Atlantic coast, including the Cape Verde Archipelago; *D. caecus* from the Mediterranean Sea, NE to SE Atlantic coast, including off West Africa.

Dispersal of chrysopetalid larvae and swimming adults to and from Senghor Seamount must largely be determined by regional and local hydrodynamic regimes. NE Atlantic water circulation near the surface does not favour transport of larvae from the European mainland towards seamounts (Surugiu et al., 2008). Mediterranean water outflow occupies the NE Atlantic at depths of around 1000 m; one branch forms an eastern boundary slope current, the other forms isolated anticyclonic vortices, with velocities of up to 30 cm s^{-1}, referred to as Meddies. Meddies consist of lenses of warm, salty water with a diameter of around 60 km that move westwards at a depth interval of 800–1400 m. Those that do not collide with seamounts may have a lifetime of up to five years (Richardson et al., 2000). Meddy structures have been inferred at Senghor from 200 m (Christiansen et al., 2010). Planktonic larvae and swimming adults of *Arichlidon reyssi* and *Dysponetus caecus* hypothetically could disperse by passive travel in the deeper currents and Meddies in a 'stepping stone' fashion along continental margins and between islands and seamounts.

Deep-sea communities are known to be strongly influenced by bathymetric gradients, although the exact controls of depth zonation remain conjectural (Carney, 2005). *Arichlidon reyssi* shares records with *Dysponetus caecus* for extreme depth ranges (from shallow to abyssal waters) within the Mediterranean and NE Atlantic. At Senghor Seamount, individuals of *Arichlidon reyssi,* morphologically identical, are found at ~100 m and also at ~1600 m. This raises the question—are we dealing with the same species or a number of cryptic species over these depth ranges?

Bik et al. (2010) found low genetic divergence across vertical depths (~2800 m) among Antarctic taxa, and identical gene sequences recorded over a 680-m depth range in another taxon within the free-living marine nematodes. Genetic analyses suggest the same species is present between 400- and 1800-m depths in a poeobiid polychaete species off Central California (K. Osborn, pers. comm.), and results for cryptic species of phyllodocid polychaetes on the NE Atlantic continental shelf of between <100 m and >1000 m confirmed shallow and deep forms represented different species (Nygren et al., 2010).

DNA studies of *Arichlidon reyssi* and *Dysponetus caecus* benthic populations at different depths would help to resolve: (i) whether the same species has the ability to live and move between areas of very different depths; (ii) whether this is evidence of the existence of different species belonging to a number of clades that may be sympatric at different depths; or (iii) whether these are distinct species living at different depths with no apparent morphological distinguishing characters to separate them.

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A graphically illustrated glossary of polychaete terminology: invasive species of Sabellidae, Serpulidae and Spionidae

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Abstract Wong, E., Kupriyanova, E.K., Hutchings, P., Capa, M., Radashevsky, V.I. and ten Hove, H.A. 2014. A graphically illustrated glossary of polychaete terminology: invasive species of Sabellidae, Serpulidae and Spionidae. *Memoirs of Museum Victoria* 71: 327–342.

A well-illustrated glossary supports the study of polychaete anatomy and systematics, as well as aiding species identification, a need that emerged within the shipping and aquaculture industries over recent decades. Sabellidae, Serpulidae and Spionidae are polychaete families that most often include species that are translocated globally through ship fouling, ballast water or aquaculture trade. Accurate identifications are crucial since these translocations have significant ecological and commercial implications and also for phylogenetic and other biological studies. Using digital illustrations of specimens (deposited predominantly at the Australian Museum in Sydney), a glossary has been developed for these three families with the aim of standardising terminologies. Complete-focus images were generated with Helicon Focus 5.3 Pro software from multiple image layers. The definitions have been explained specific to families and illustrated with these images, thus creating the first comprehensive, digitally illustrated glossary of polychaete terminology.

Keywords invasive, biofouling, biosecurity, identification key, digital photographs, Australia

Introduction

The identification of polychaetes, as with all invertebrate groups, requires an understanding of both the morphological features and the terminologies used to describe these features. Therefore, a glossary underpins the study of the systematics of a particular group. Soon after its publication, the glossary of the 'pink' book (Fauchald, 1977) became a standard reference for terms used in systematic polychaete literature. However, terminology used for polychaete features has varied greatly among authors, resulting in confusion that has never been resolved, even within individual families (e.g. Nogueira *et al.*, 2010, for Terebellidae; Capa and Murray, 2009, and Capa *et al.*, 2011a and 2011b, for Sabellidae; ten Hove and Kupriyanova, 2009, for Serpulidae; Light, 1978, and Radashevsky, 2012, for Spionidae). Moreover, the terms used for homologous structures may differ considerably between families, while identical terms are sometimes used for features with different origins (e.g. 'branchia' in Serpulidae and Spionidae)—hence the potential for confusion.

In recent decades, the need for polychaete identification has arisen among the shipping and port management industries as a result of increasing global trade, as well as within the aquaculture industry. Environmental consultants, biologists and quarantine officers are required to examine ship hulls and wharves in ports and marinas for anthropogenically translocated organisms, including polychaetes. Invasions of pest species threaten local marine communities and biodiversity, generating substantial

losses for the aquaculture, shipping and tourism industries (Holloway and Keough, 2002; Bax *et al*., 2003; Çinar, 2012). The polychaete families Serpulidae, Sabellidae and Spionidae collectively comprise 40% of the translocated polychaetes worldwide (Çinar, 2012), and some of these are listed as pest species (DAFF, 2012) as they can have considerable impact on native ecosystems, including the potential to displace local species (Çinar *et al*., 2005; Çinar, 2012). For many species, the impacts are yet to be studied.

The obvious need for a well-illustrated digital guide for non-specialists resulted in the Invasive Polychaete Identifier (Kupriyanova *et al*., 2013) that was developed at the Australian Museum with the aim of enabling identification of Australian native and invasive (or potentially invasive) polychaetes. This guide includes a glossary that is linked to the terms used in the text. The approach taken by the guide is comprehensive visualisation for identifications of sabellid, serpulid and spionid species. Museum specimens were photographed through a Leica MZ16 dissection microscope fitted with a Spot Flex 15.2 camera. Some specimens were stained with methylene blue or methyl green to increase contrast and thus visually enhance important diagnostic features. Slides were made of chaetae of some species. Helicon Focus 5.3 Pro software was used to create completely focused images by integrating the layers of partially focused images captured.

There have been previous attempts to standardise definitions within each of the three families under consideration. The influential taxonomic revision of Sabellidae by Fitzhugh (1989) has for years been the source of terminology for this family, and Capa *et al*. (2011a) recently reviewed the terminology of most sabellid morphological features. Ten Hove and Kupriyanova (2009) reviewed the state of taxonomy in Serpulidae (not including, however, the subfamily Spirorbinae) and provided a discussion of morphology and a glossary for the family. Most recently, Radashevsky (2012) reviewed the morphology of Spionidae and the terms used in this family. As a next step towards easier communication of taxonomic information, here we provide the first fully illustrated glossary of the polychaete terms that are specific to these three families (Sabellidae, Serpulidae and Spionidae). While we have attempted to standardise terms, we are not implying that structures with the same name are necessarily homologous structures, and in many cases detailed developmental studies are required to ascertain this. The terminologies pertaining to general biology and systematics are not covered, as it is expected that users can refer to standard textbooks and literature (e.g. Beesley *et al*., 2000; Rouse and Pleijel, 2001) if they are not already equipped with this knowledge.

GLOSSARY

A

abdomen (Sabellidae and Serpulidae): body region posterior to the thorax; recognised by notopodial (dorsal) uncini and neuropodial (ventral) chaetae (fig. 1a).

accessory gills (Spionidae): see **branchiae**.

acicular spine (Spionidae): straight thick chaetae in notopodia of posterior segments (fig. 1b).

acicular uncinus (pl. acicular uncini) (Sabellidae): hook-shaped uncinus with a poorly developed breast and a long handle (fig. 1c).

anal depression (Sabellidae): dorsoventrally flattened expansion of posterior abdominal segments, accompanied in some species by lateral flanges (fig. 1d).

anterior peristomial ring (Sabellidae): anterior part of the peristomium, attached to the radiolar lobe; ventral anterior lobe can be triangular or rounded (fig. 1e).

apron (Serpulidae): membranous flap formed by thoracic membranes joined ventrally past the last thoracic chaetigers (fig. 1f).

avicular uncinus (pl. avicular uncini) (Sabellidae): Z-shaped uncinus with well-developed breasts and a handle (fig. 1g).

B

bayonet chaetae (Serpulidae): special collar chaetae with 1 or 2 (sometimes more) large proximal teeth at the base of a distal limbate zone (fig. 1h).

bayonet chaetae (Sabellidae): small, thin and slightly bent, narrowly hooded (see **limbate chaetae**) thoracic and abdominal chaetae (fig. 1i).

bifurcate: divided into 2 parts or branches.

bilimbate: chaetae with a hood (limbus) visible on both sides of the shaft; see **limbate chaetae** and **broadly hooded**.

branchiae (Spionidae): paired body appendages on segments, provided with blood loop for respiration (fig. 1j). N.B., different from radiolar crown of Sabellidae and Serpulidae.

breast (Sabellidae and Serpulidae): rounded part of an uncinus; located below the main fang in Sabellidae or anterior fang (peg) in Serpulidae (fig. 2a). Uncini with well-developed breasts are avicular (Sabellidae).

broadly hooded (Sabellidae and Serpulidae): hooded capillaries with the distal hood (limbus) enlarged on both sides of the shaft and appearing bilimbate under the compound microscope (fig. 2b).

C

capillary chaetae: slender, often long, chaetae, tapering to a fine point; the term has been used as a collective term for elongate, needle-like or hair-like chaetae of otherwise variable shape and ontogeny (fig. 2c).

caruncle (Spionidae): a dorsal extension of the prostomium, taking the form of an elevation or a distinct crest separating the nuchal organs one from another (fig. 2d).

chaeta (pl. chaetae) (hence Polychaeta, 'with many hairs'): chitinous bristle protruding from an epidermal pocket in the body wall (fig. 2e).

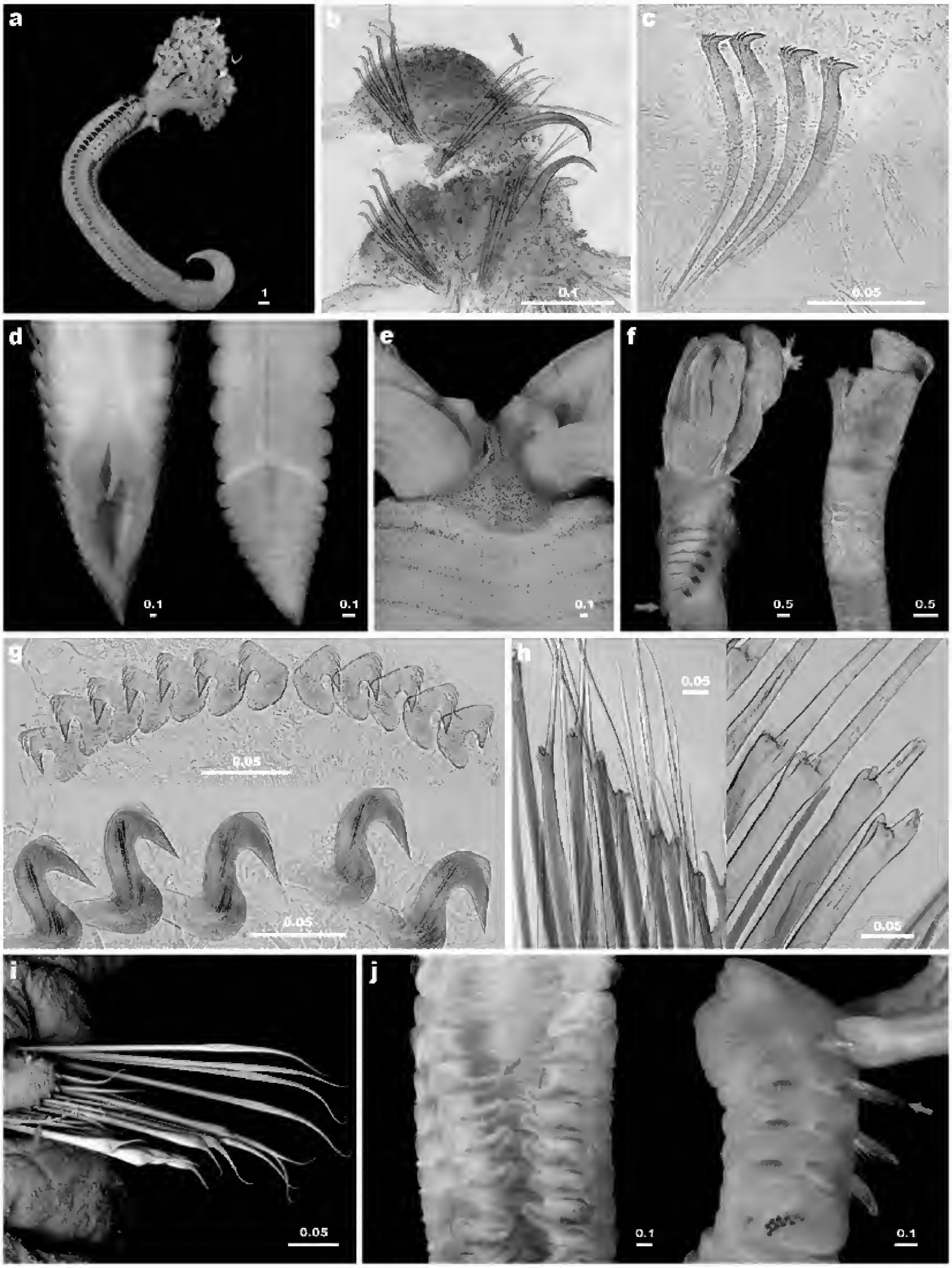

Figure 1. (a) *Bispira manicata* with abdomen region highlighted red. (b) Posterior notopodia of *Boccardiella bihamata* stained with methyl green; arrow points to acicular spine. (c) Acicular thoracic uncini of *Euchone limnicola*. (d) Ventral anal depression in *Euchone variabilis* (left, stained with methylene blue) with lateral flanges, and in *Euchone limnicola*, without flanges. (e) Collar region/base of radiolar crown in *Myxicola infundibulum* stained with methylene blue; anterior peristomial ring highlighted red. (f) Lateral view of *Spirobranchus tetraceros* (left) and ventral view of *Spirobranchus kraussii* (right), both stained with methylene blue; arrows point to apron. (g) Z-shaped avicular thoracic uncini of *Laonome triangularis* (above) and *Bispira manicata* (below, stained with methyl green). (h) Bayonet collar chaetae in *Serpula jukesi*, stained with methyl green. (i) Bayonet thoracic chaetae in *Jasmineira* sp. (j) Arrows point to branchiae in *Boccardia chilensis* (left and right specimens stained with methylene blue and methyl green, respectively). All scales in mm.

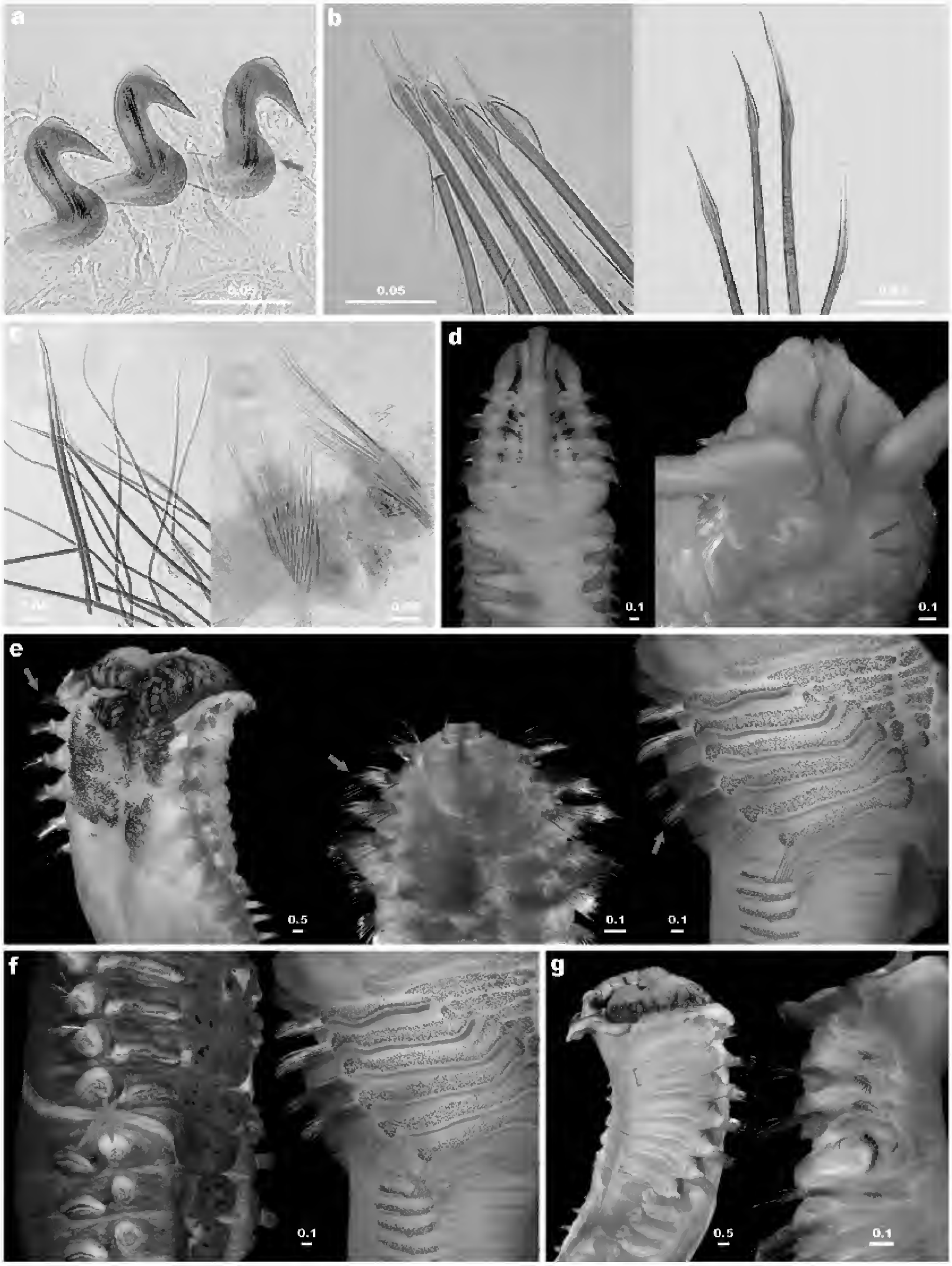

Figure 2. (a) Thoracic uncini of *Bispira manicata*, stained with methyl green; arrow points to breast of uncinus. (b) Broadly hooded thoracic chaetae of *Euchone limnicola* and collar chaetae of *Laonome calida*. (c) Capillary chaetae from collar of *Spirobranchus taeniatus* (on left, stained with methyl green) and chaetiger 1 of *Boccardia proboscidea* (on right). (d) Dorsal view of anterior ends of *Polydora haswelli* (on left, stained with methyl green) and *Boccardia proboscidea* (on right); arrows point to caruncle. (e) Arrows point to thoracic chaetae of (left to right, respectively) *Bispira porifera*, *Boccardiella bihamata* (stained with methyl green) and *Spirobranchus cariniferus* (stained with methylene blue). (f) Arrows demonstrate chaetal inversion in *Branchiomma bairdi* (left) and *Spirobranchus cariniferus* (right). (g) Lateral views of thoracic regions of *Bispira porifera* (left) and *Polydora haswelli* (right, stained with methyl green); each bracket indicates 1 chaetiger. All scales in mm.

chaetal inversion (Sabellidae and Serpulidae): the thorax bears chaetae dorsally (in notopodia) and uncini ventrally (in neuropodia); while in the abdomen the position of the chaetae and uncini is reversed (fig. 2f).

chaetiger: segment bearing chaetae (fig. 2g).

cirrus (pl. cirri): soft tactile appendage, usually on parapodia, peristomium and pygidium (fig. 3a).

cirriform (Spionidae): bearing cirri (fig. 3a).

collar (Sabellidae and Serpulidae): a more or less encircling membranous flap projecting from the peristomium and, in some cases, covering the base of the radiolar crown (fig. 3c).

collar chaetae (Sabellidae and Serpulidae): notochaetae of the first (collar) chaetiger not accompanied by neuropodial uncini (fig. 3d).

collar segment (Sabellidae and Serpulidae): first chaetiger, often bearing a membranous collar and notochaetae (see **collar chaetae**), but lacking uncini (fig. 3e).

companion chaetae (Sabellidae): chaetae with a basal shaft and a distal hood, arranged in a single row, parallel to the row of thoracic uncini (fig. 3b).

companion chaetae (Spionidae): short capillary chaetae, usually distally bilimbate, accompanying heavy falcate spines on segment 5 in polydorins (members of the tribe Polydorini) (fig. 5g).

constriction (Serpulidae): narrowing of the opercular peduncle at base of opercular funnel or ampulla (fig. 3f).

constriction (Spionidae): narrowing of the upper part of hook shaft (fig. 3g).

D

distal wings (Serpulidae): paired lateral outgrowths of the peduncle located just below the operculum (fig. 3h).

dorsal: pertaining to, or situated at, the back (dorsum) (fig. 4a).

dorsal lips (Sabellidae): paired rounded lappets on dorsal margin of mouth; used for feeding, tube building, and sorting the particles collected by the radiolar crown (fig. 4b).

dorsal radiolar appendages (Sabellidae): modified radioles fused to dorsal lips (fig. 4b).

F

falcate spines (Spionidae): chaetae resembling mammalian canine teeth; characteristically present in the posterior row notochaetae of segment 5 in polydorins (members of the tribe Polydorini) (fig. 4c).

faecal groove (Sabellidae and Serpulidae): ciliated channel running along the body and used for directing the faeces from the anus to the anterior tube opening (fig. 4d).

faecal groove inversion (Sabellidae and Serpulidae): change in the position of the ciliated groove (used to direct faeces from the anus to the tube mouth): it runs ventrally in the abdomen, passing between the last thoracic notopodia and the first abdominal neuropodia, and becomes dorsal in the thorax (fig. 4e).

flat trumpet-shaped chaetae (Serpulidae): in profile resembling a hollow trumpet, with distal expanded part edged with 2 rows of teeth. However, examination with SEM shows that these chaetae are flat, with a single row of acute marginal teeth (fig. 4f).

funnel (Serpulidae): inverted, cone-like proximal part of the operculum in *Hydroides*, and the entire operculum in *Serpula* (fig. 4g).

G

glandular girdle (Sabellidae): complete or incomplete pale ridge around the first or second chaetiger (fig. 4h).

H

handle (Sabellidae): posterior elongated extension of an uncinus; always embedded in body wall (fig. 4i).

hood (preferred term for Sabellidae): distal extension of capillary chaetae, appearing as a flat, longitudinal flange under the compound microscope, but made of tightly packed microfibrils as seen under SEM (fig. 5a). N.B., the same as limbus in Serpulidae.

hood (Spionidae): a thin sheath surrounding the distal dentate end of hooks (fig. 5b). N.B., not the same as hood in Sabellidae.

hooded chaetae (preferred term for Sabellidae): capillary chaetae with hood. N.B., the same as limbate chaetae in Serpulidae.

hooded chaetae (Spionidae): hooked chaetae with hood (see **hood** for Spionidae). N.B., not the same as hooded chaetae in Sabellidae.

hooks (Spionidae): distally curved chaetae used to hold individual inside the burrow or tube (fig. 5c); also see **uncinus**, which can be hook-shaped in Sabellidae.

I

inter-radiolar membrane (Sabellidae and Serpulidae): membrane connecting basal parts of radioles (fig. 5d).

inter-ramal eyespots (Sabellidae): simple eyes located between the rami (notopodia and neuropodia) in both thoracic and abdominal segments (fig. 5e).

K

keel (Serpulidae): outer longitudinal prominent ridge running along the calcareous tube length (fig. 5f).

L

lappet: lobe or flap-like projection.

lateral: located on the side.

Figure 3. (a) Cirriform pygidium of *Pygospio elegans*, stained with methyl green; arrow points to a cirrus. (b) Companion chaetae (arrows) as parallel row anterior to thoracic uncini in *Sabella spallanzanii* (left) and *Bispira manicata* (right, stained with methylene blue). (c) Arrows point to collar flaps in *Laonome calida* (left) and *Spirobranchus cariniferus* (right), both stained with methylene blue. (d) Collar/thoracic regions of *Laonome triangularis* (left, stained with methylene blue) and *Ficopomatus enigmaticus* (right); arrows point to collar chaetae. (e) Collar segments indicated by different arrows in (left to right, respectively) *Branchiomma galei*, *Laonome triangularis* (stained with methylene blue) and *Spirobranchus cariniferus* (stained with methylene blue). (f) Constrictions, indicated by arrows, occurring below funnels in opercula of *Hydroides malleolaspinus* and *Hydroides minax*. (g) Constriction, indicated by arrow, in upper shaft of neuropodial hooks of *Polydora uncinata*. (h) Opercula of *Spirobranchus polytrema*, *S. cariniferus* and *S. tetraceros* (left to right, respectively); arrows indicate distal wings. All scales in mm.

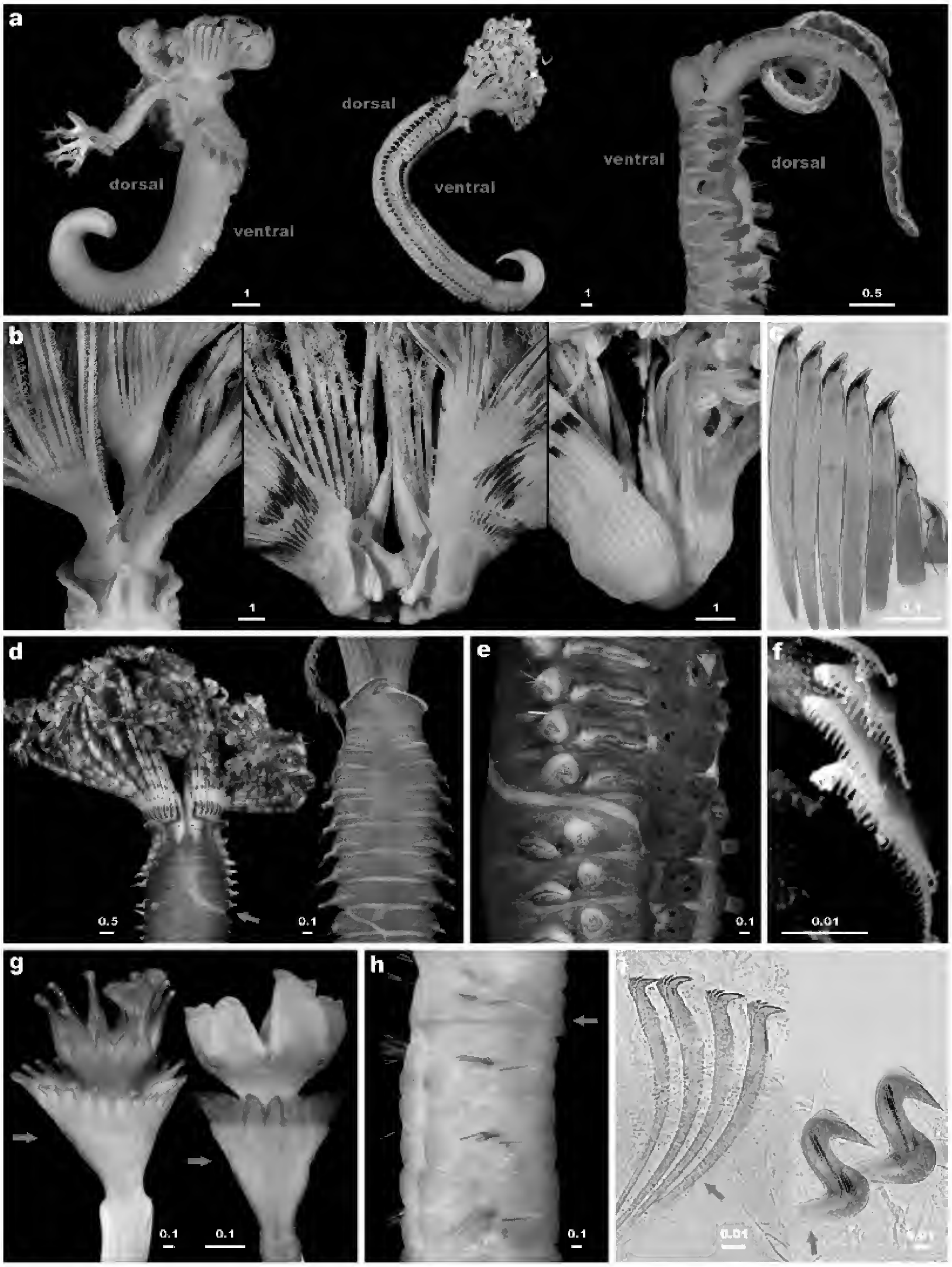

Figure 4. (a) Dorsal/ventral sides illustrated on examples of (left to right, respectively) Serpulidae (*Spirobranchus tetraceros*), Sabellidae (*Bispira manicata*) and Spionidae (*Polydora haswelli*, stained with methyl green). (b) Arrows indicating paired dorsal radiolar appendages, fused to dorsal lips, in (left to right, respectively) *Sabella spallanzanii*, *Bispira porifera* and *Bispira manicata*. (c) Falcate spines in notochaetae on chaetiger 5 of *Polydora uncinata*. (d) Anterior regions of *Branchiomma bairdi* (dorsal view) and *Laonome calida* (ventral view, stained with methylene blue); arrows indicate faecal grooves. (e) Faecal groove inversion in *Branchiomma bairdi*: faecal groove runs ventrally in abdomen and dorsally in thorax. (f) SEM image of flat trumpet-shaped abdominal chaetae in *Serpula columbiana*. (g) Arrows indicate opercula funnels in *Hydroides malleolaspinus* and *Hydroides tuberculatus*. (h) Arrow indicates glandular girdle on chaetiger 2 of *Euchone variabilis*, stained with methylene blue. (i) Arrows indicate handles of acicular thoracic uncini in *Euchone limnicola* (left) and avicular thoracic uncini in *Bispira manicata* (right, stained with methyl green). All scales in mm.

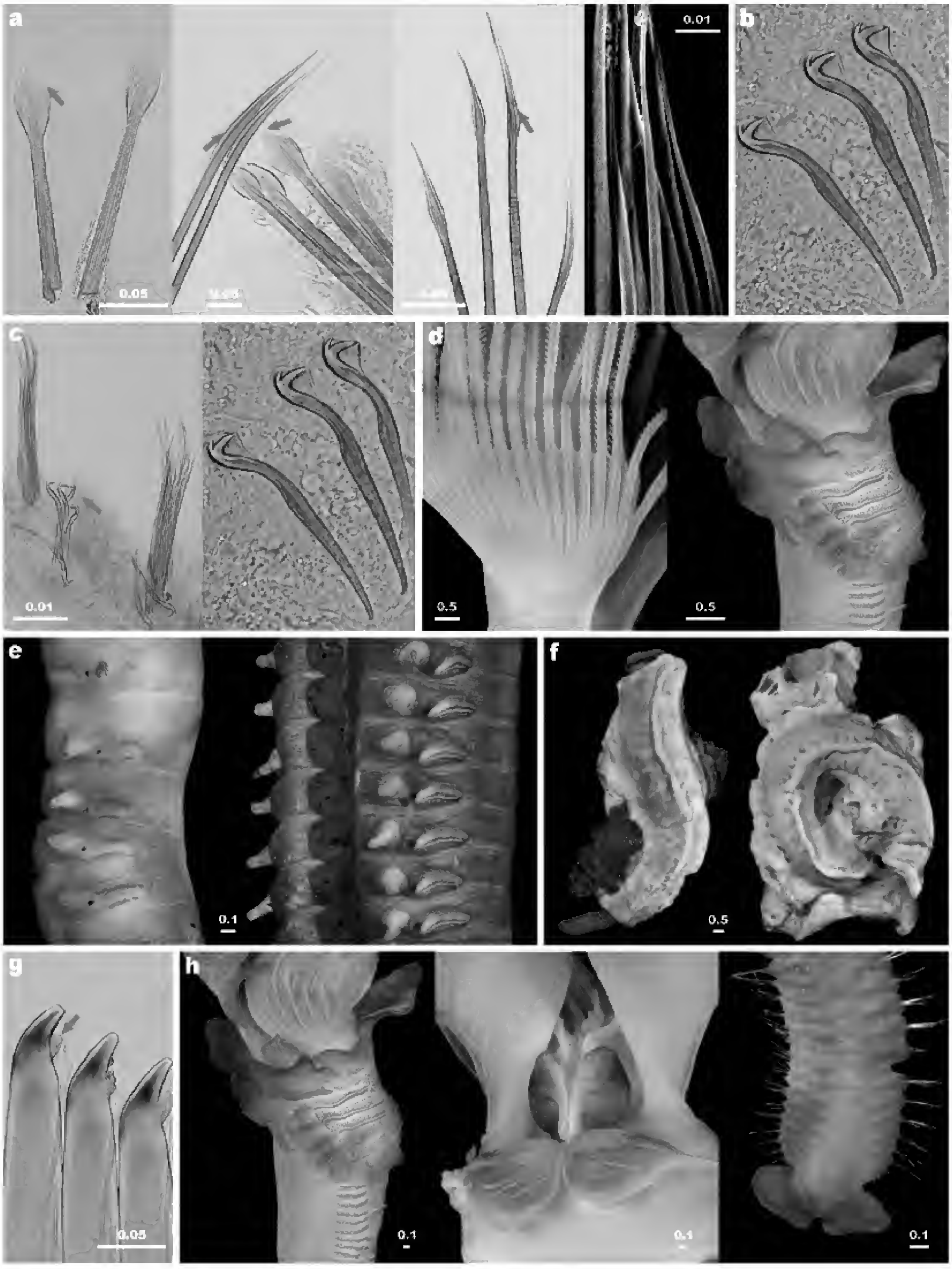

Figure 5. (a) Arrows indicate hood on collar chaetae of *Laonome triangularis* (left 2 images), *Laonome calida* and thoracic chaetae of *Crucigera websteri* (SEM image). (b) Arrow indicates hood in neuropodial hooks of *Polydora uncinata*. (c) Hooks in posterior neuropodia of *Polydora uncinata*. (d) Radioles proximally connected by inter-radiolar membranes (arrows) in *Sabella spallanzanii* and *Spirobranchus cariniferus* (stained with methylene blue). (e) Inter-ramal eyes (arrows) located between notopodia and neuropodia of *Branchiomma galei* and *Branchiomma bairdi*. (f) Calcareous tubes of *Spirobranchus cariniferus* (left) and *Spirobranchus kraussii* (right); arrows indicate keels on tube. (g) Falcate spines in notopodia of chaetiger 5 of *Polydora uncinata*; arrow indicates lateral flange. (h) Lobate condition in collars of (left to right, respectively) *Spirobranchus cariniferus* (stained with methylene blue), *Sabella spallanzanii* and pygidium of *Polydora ciliata* (stained with methyl green). Lobes highlighted red. All scales in mm.

lateral flange (Spionidae): small subdistal structure on heavy falcate spines in polydorin spionids (fig. 5g).

limbate chaetae (preferred term for Serpulidae): capillary chaetae with limbus. N.B., the same as hooded chaetae in Sabellidae.

limbus (preferred term for Serpulidae): distal extension of capillary chaetae; appearing as a flattened longitudinal flange under the compound microscope, but made of tightly packed microfibrils as seen under SEM. N.B., the same as hood in Sabellidae.

lobate: subdivided into lobes (fig. 5h).

M

main fang (Sabellidae and Serpulidae): largest fang (or tooth) of an uncinus, surmounted by row(s) of much smaller teeth (fig. 6a).

male horns (Spionidae): a pair of dorsal appendages on segment 2 in *Pygospio* males (fig. 6b).

median antenna (Spionidae): see **occipital antenna**.

N

narrowly hooded (Sabellidae): capillary chaetae with the distal hood (limbus) only on 1 side of the shaft (fig. 6c); see **limbate chaetae**.

neurochaetae: chaetae of neuropodia (fig. 6d).

neuropodium (pl. neuropodia): ventral branch or ramus of a parapodium (fig. 6d).

notochaetae: chaetae of a notopodium (fig. 6e).

notopodium (pl. notopodia): dorsal branch or ramus of a parapodium (fig. 6e).

nuchal organs: paired ciliated sensory organs on the prostomium; in Spionidae extending over dorsal side of certain anterior segments as ciliary bands, entire or metameric (fig. 6f).

nuchal papilla (Spionidae): see **occipital antenna**.

O

occipital antenna (Spionidae): a short median appendage on the prostomium (fig. 6f).

operculum (pl. opercula) (Serpulidae): tip of modified radiole used to plug the tube when the worm is retracted (fig. 6g).

opercular endplate (Serpulidae): terminal reinforcement of operculum, often chitinous or calcareous (fig. 7a).

P

paleate (Sabellidae): broadly hooded (bilimbate) capillaries with the shaft not reaching the tip of the chaetae.

palmate: having lobes radiating from a common point (fig. 7b).

palps: a pair of feeding and/or tactile appendages arising from the head or anterior end of body (fig. 7c).

parapodium (pl. parapodia): fleshy lateral projection from a body segment; usually bearing chaetae (fig. 7d).

peduncle (Serpulidae): modified radiole bearing the operculum (fig. 7e).

peduncular wings (Serpulidae): see **distal wings**.

peristome (Serpulidae): collar-like widening of tube; former tube mouth (fig. 7f).

peristomium: anterior body region surrounding the mouth and located posterior to and/or below the prostomium (fig. 7g).

pinnules (Sabellidae and Serpulidae): small ciliated paired outgrowths located along from the inner edge of the radioles, giving each radiole a feathery appearance (fig. 7h).

prostomium: anteriormost presegmental region of body; usually bearing radioles and sensory organs such as palps, antennae, nuchal organs and eyes.

posterior peristomial ring (Sabellidae and Serpulidae): posterior part of the peristomium; may bear a membranous collar.

pseudoperculum (pl. pseudopercula) (Serpulidae): modified radiole, generally without pinnules; can develop into a new functional operculum when the functional operculum is lost (fig. 7i).

pygidium: postsegmental terminal body part surrounding the anus (fig. 7j).

R

radiolar crown (Sabellidae and Serpulidae): anterior part extended outside the tube and used for feeding and respiration; of prostomial origin and made of pinnulated radioles attached to radiolar lobes around the mouth (fig. 8a).

radiolar eyes (Sabellidae and Serpulidae): ocelli found in the radiolar crown; can vary in number, arrangement and structure (fig. 8b).

radiolar flanges (Sabellidae): paired, lateral membranous extensions along outer margins of radioles (fig. 8c).

radiolar lobes (Sabellidae and Serpulidae): proximal part of the radiolar crown attached to the anterior end of the body; generally arranged as 2 semicircles, 1 on each side of the mouth, but forming spirals in some species (fig. 8d).

radioles (Sabellidae and Serpulidae): filaments making up the radiolar crown; attached to the radiolar lobes and bearing rows of paired ciliated pinnules (fig. 8e).

radius (pl. radii) (Serpulidae): radial projection of the funnel (genera *Hydroides* and *Serpula* only) (fig. 8f).

ramus: a branch.

rasp-shaped uncini (Sabellidae and Serpulidae): with 2 or more rows of teeth (fig. 8g).

recurved spines (Spionidae): heavy chaetae with distal parts bent backwards, found in notopodia of posterior segments (fig. 8h).

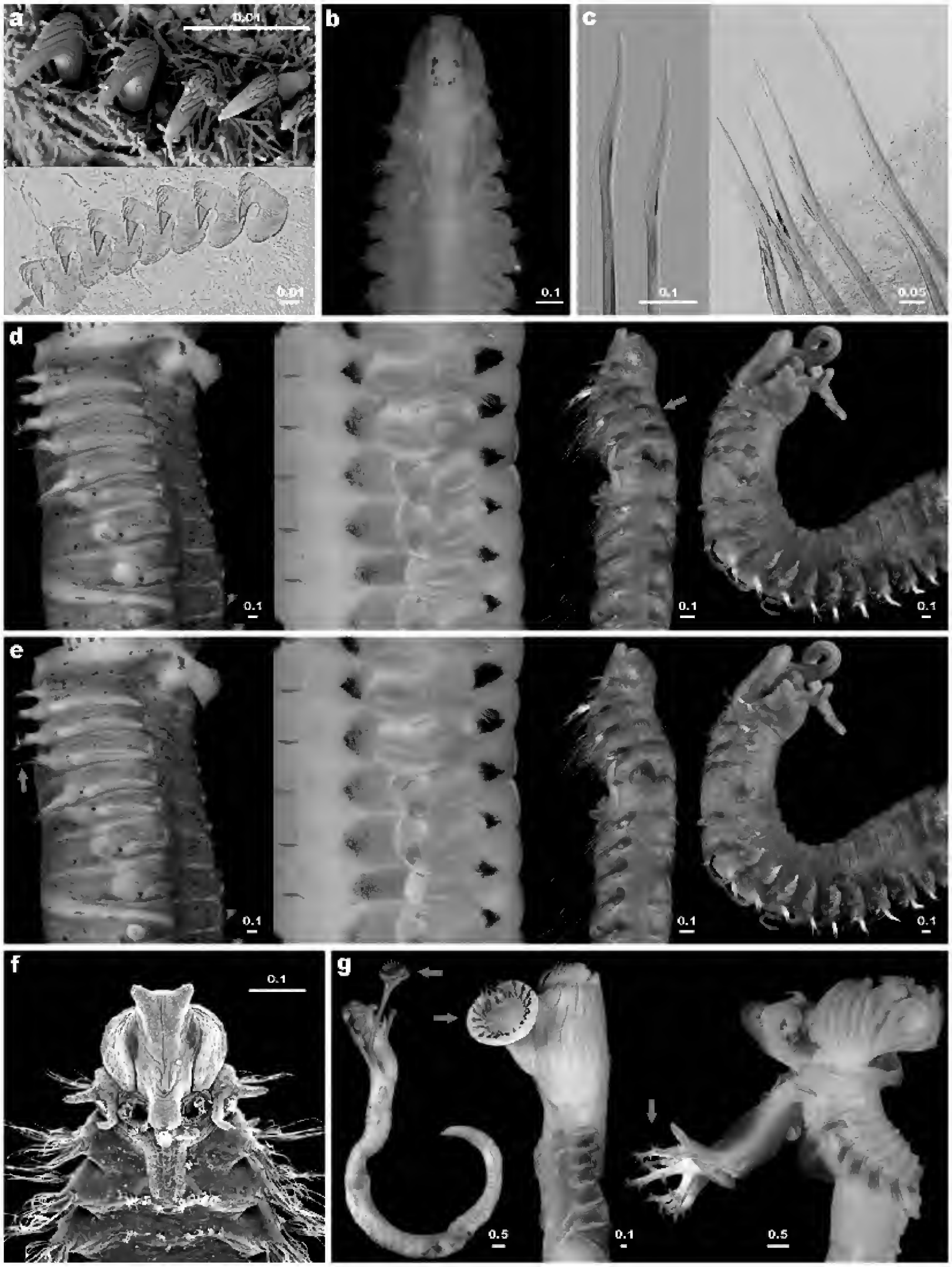

Figure 6. (a) Thoracic uncini of *Desdemona aniara* (above, SEM image) and *Laonome triangularis* (below); arrows indicate main fangs. (b) A pair of dorsal horns (indicated by arrow) on chaetiger 2 of a male of *Pygospio elegans*. (c) Narrowly hooded thoracic chaetae of *Sabellastarte australiensis*. (d) Thoracic neuropodia highlighted red on (left to right, respectively) *Branchiomma bairdi*, *Bispira manicata*, *Boccardiella bihamata* (stained with methyl green) and *Boccardia proboscidea* (stained with methyl green); arrows indicate neurochaetae. (e) Thoracic notopodia highlighted red on (left to right, respectively) *Branchiomma bairdi*, *Bispira manicata*, *Boccardiella bihamata* (stained with methyl green) and *Boccardia proboscidea* (stained with methyl green); arrows indicate notochaetae. (f) SEM image of dorsal anterior end of *Polydora cornuta*. Red arrows indicate a pair of nuchal organs; outlined arrow indicates occipital antenna. (g) Arrows indicate opercula of (left to right, respectively) *Hydroides norvegicus* (stained with methylene blue), *Ficopomatus enigmaticus* and *Spirobranchus tetraceros*. All scales in mm.

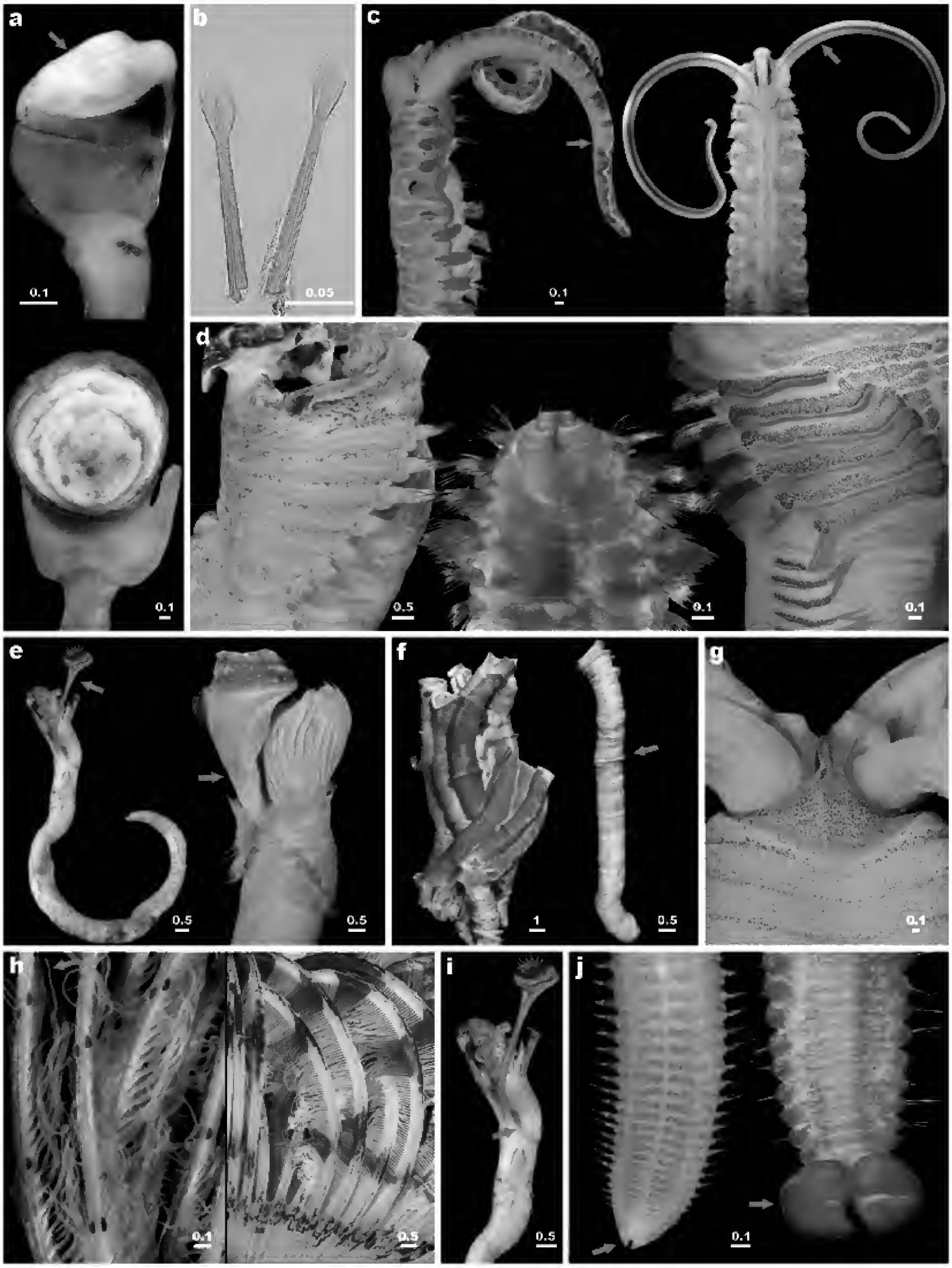

Figure 7. (a) Operculum of *Spirobranchus minutus* (above) and *Spirobranchus kraussii* (below); arrows indicate opercular endplate. (b) Paleate collar chaetae of *Laonome triangularis*. (c) Arrows indicate palps of *Polydora haswelli* (stained with methyl green) and *Boccardia proboscidea* (live specimen). (d) Parapodia highlighted red in (left to right, respectively) *Sabellastarte australiensis*, *Boccardiella bihamata* (stained with methyl green) and *Spirobranchus cariniferus* (stained with methylene blue). (e) Arrows indicate peduncle of *Hydroides norvegicus* (stained with methylene blue) and *Spirobranchus cariniferus*. (f) Tubes of *Ficopomatus enigmaticus* and *Ficopomatus uschakovi*; arrows indicate peristomes. (g) Collar region/base of radiolar crown in *Myxicola infundibulum* stained with methylene blue; peristomium highlighted red. (h) Radioles of *Bispira serrata* and *Bispira porifera*; arrows indicate individual pinnules. (i) Anterior end of *Hydroides norvegicus* (stained with methylene blue); arrow indicates pseudoperculum. (j) Arrows indicate pygidium of *Bispira serrata* and *Boccardia polybranchia* (stained with methyl green). All scales in mm.

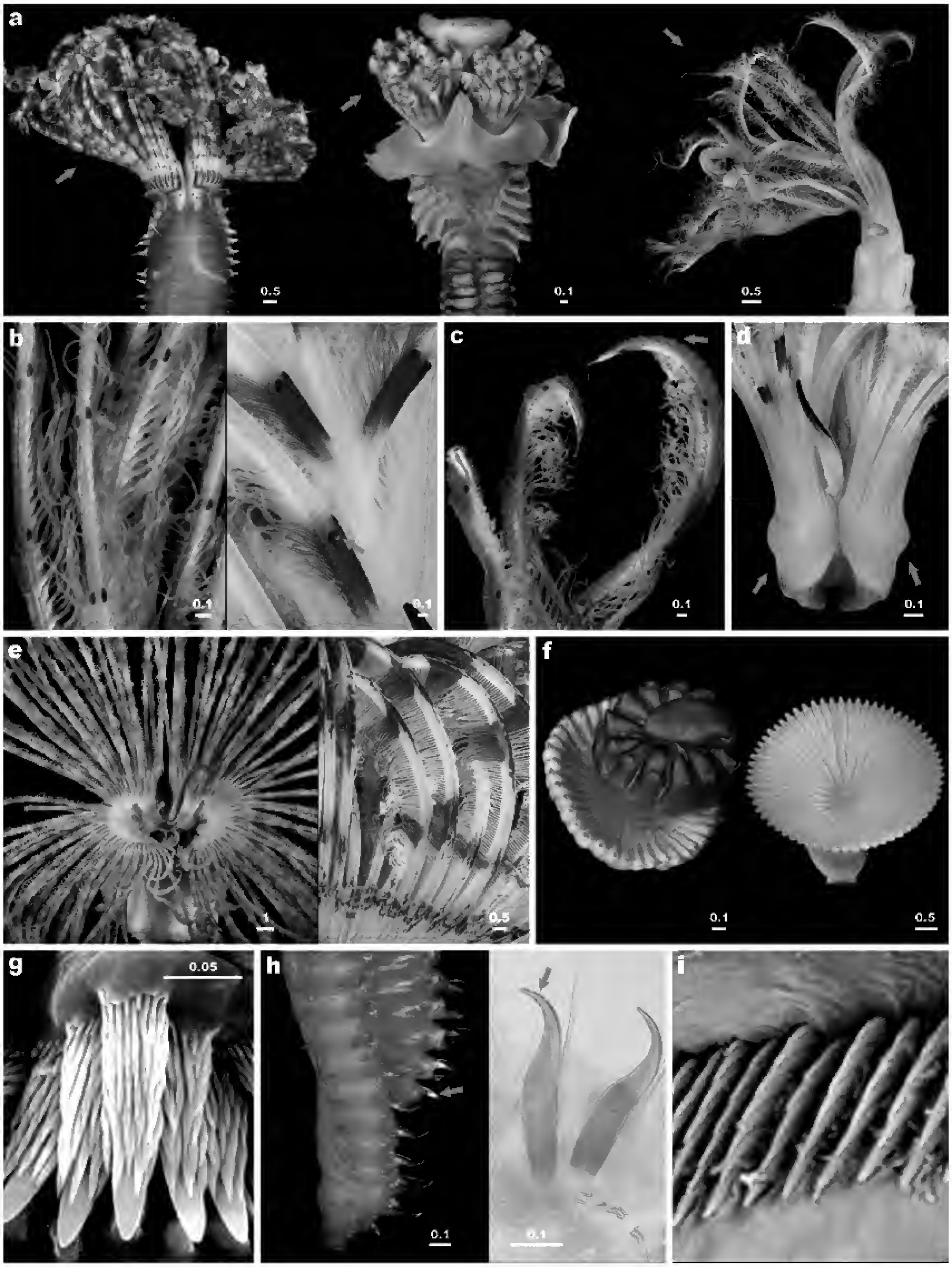

Figure 8. (a) Radiolar crowns in (left to right, respectively) *Branchiomma bairdi*, *Spirobranchus kraussii* and *Euchone variabilis*. (b) Radioles of *Bispira serrata* and *Bispira manicata*; arrows indicate radiolar eyes. (c) Arrow indicates radiolar flange on *Bispira serrata*. (d) Radiolar crown of *Bispira manicata*, consisting of 2 radiolar lobes (arrows). (e) Radiole filaments of *Sabellastarte australiensis* and *Bispira porifera*. (f) Single radius highlighted red in *Hydroides brachyacanthus* and *Serpula jukesii*. (g) SEM image of rasp-shaped posterior abdominal uncini in *Serpula columbiana*. (h) Recurved spines in posterior notopodia of *Boccardiella bihamata* (on left, stained with methyl green) and *Polydora uncinata* (on right). (i) SEM image of saw-shaped thoracic uncini on *Serpula columbiana*. All scales in mm.

S

saw-shaped uncini (Serpulidae): with only 1 row of teeth (fig. 8i).

sedentary: attached to a surface and not moving freely.

segment: 1 of the serially repeated units comprising the trunk; often separated internally by septae or dissepiments (fig. 9a).

shaft: proximal smooth part of chaetae, partly embedded in the tissue; also see **handle**.

spine-like chaetae (Sabellidae): narrowly hooded capillaries (also see **limbate chaetae**). N.B., not the same as falcate spines and recurved spines of Spionidae.

spinules (Serpulidae): each of the tubercular or tooth-like projections of a spine in the verticil of the genus *Hydroides* (fig. 9b). By their position relative to the axis, spinules may be internal, lateral or external. By their position along the spine, spinules may be proximal, medial or distal.

***Spirobranchus*-type chaetae** (Serpulidae): special collar chaetae with a 'fin' positioned below the distal limbus (hood) and consisting of numerous tiny hair-like spines (fig. 9c).

stylodes (Sabellidae and Serpulidae): outward projections from the outer margin of radioles; can be digitiform (cylindrical or finger-like), strap-like (flattened) or palmate (branched and flattened); always paired in Sabellidae, unpaired in Serpulidae (fig. 9d).

T

thoracic membrane (Serpulidae): thin folds on both sides of thorax, extending from dorsal part of collar to lateral and/or ventral side of posterior thorax (fig. 9e).

thorax: anterior region of the body behind the head (fig. 9f).

tonguelet (Serpulidae): special form of lappet, between dorsolateral and ventral lobes of the collar in some serpulid genera (fig. 9g).

torus (pl. tori) (Sabellidae and Serpulidae): transverse elevation of parapodium surrounding the uncini (fig. 10a).

triangular depression (Serpulidae): depressed area between thoracic uncinigerous tori which gradually approach and almost touch one another posteriorly and ventrally (fig. 10b).

true trumpet-shaped chaetae (Serpulidae): distally hollow chaetae, with 2 parallel rows of sharp denticles, extending into a long lateral spine (fig. 10c).

tube: protective structure completely enclosing the body in some polychaete families; made of mucus often covered by sediment particles (Sabellidae, Spionidae) or calcium carbonate (Serpulidae, exceptionally Sabellidae) (fig. 10d).

U

uncinigerous (Sabellidae and Serpulidae): bearing uncini.

uncinus (pl. uncini) (Sabellidae and Serpulidae): small modified hook-shaped or comb-shaped chaeta deeply embedded into tissue, with only its dentate edge protruding from the body wall; uncini usually arranged in tori in transverse elevated rows (fig. 10e).

V

ventral: lower or underside of the body; side of the polychaete body bearing the mouth.

ventral lips (Sabellidae): membranous lappets on both lateroventral sides of mouth (fig. 10f).

ventral radiolar appendages (Sabellidae): modified radioles generally lacking pinnules; located on the ventral edge of the radiolar lobes.

ventral sacs (Sabellidae): vesicles filled with sediment used for tube building; located between the radiolar lobes (fig. 10g).

ventral shields (Sabellidae and Serpulidae): epidermal glandular areas on ventral side of the thorax; well-defined or diffused (fig. 11a).

verticil (Serpulidae): distal part (usually a crown of chitinous spines) of operculum in *Hydroides* (fig. 11b).

verticil spine (Serpulidae): radial elements together forming the verticil in *Hydroides* (fig. 11b).

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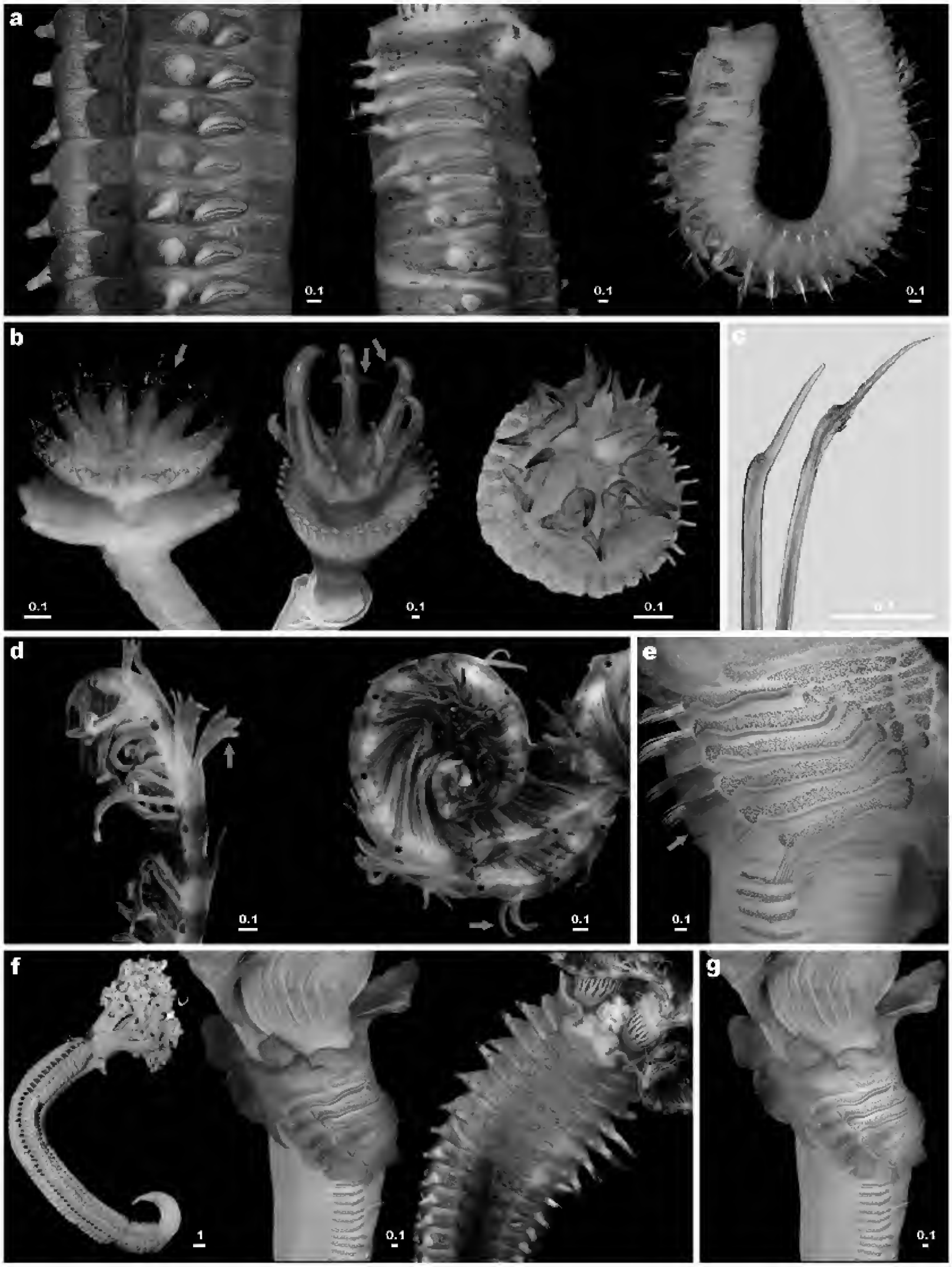

Figure 9. (a) Single segments of *Branchiomma bairdi* (left 2 images) and *Polydora haswelli* (stained with methyl green) highlighted red. (b) Arrows indicate spinules on opercula of (left to right, respectively) *Hydroides elegans*, *H. heteroceros* and *H. tambalagamensis*. (c) *Spirobranchus*-type collar chaetae from *Spirobranchus tetraceros* (stained with methyl green). (d) Radioles of *Branchiomma galei* and *Branchiomma bairdi*; arrows indicate stylodes (palmate in *B. galei* and simple in *B. bairdi*). (e) Arrow indicates thoracic membrane of *Spirobranchus cariniferus*, stained with methylene blue. (f) Thorax regions (highlighted red) of (left to right, respectively) *Bispira manicata*, *Spirobranchus cariniferus* (stained with methylene blue) and *Branchiomma bairdi*. (g) Arrow indicates tonguelet of *Spirobranchus cariniferus* (stained with methylene blue), partially covered by collar. All scales in mm.

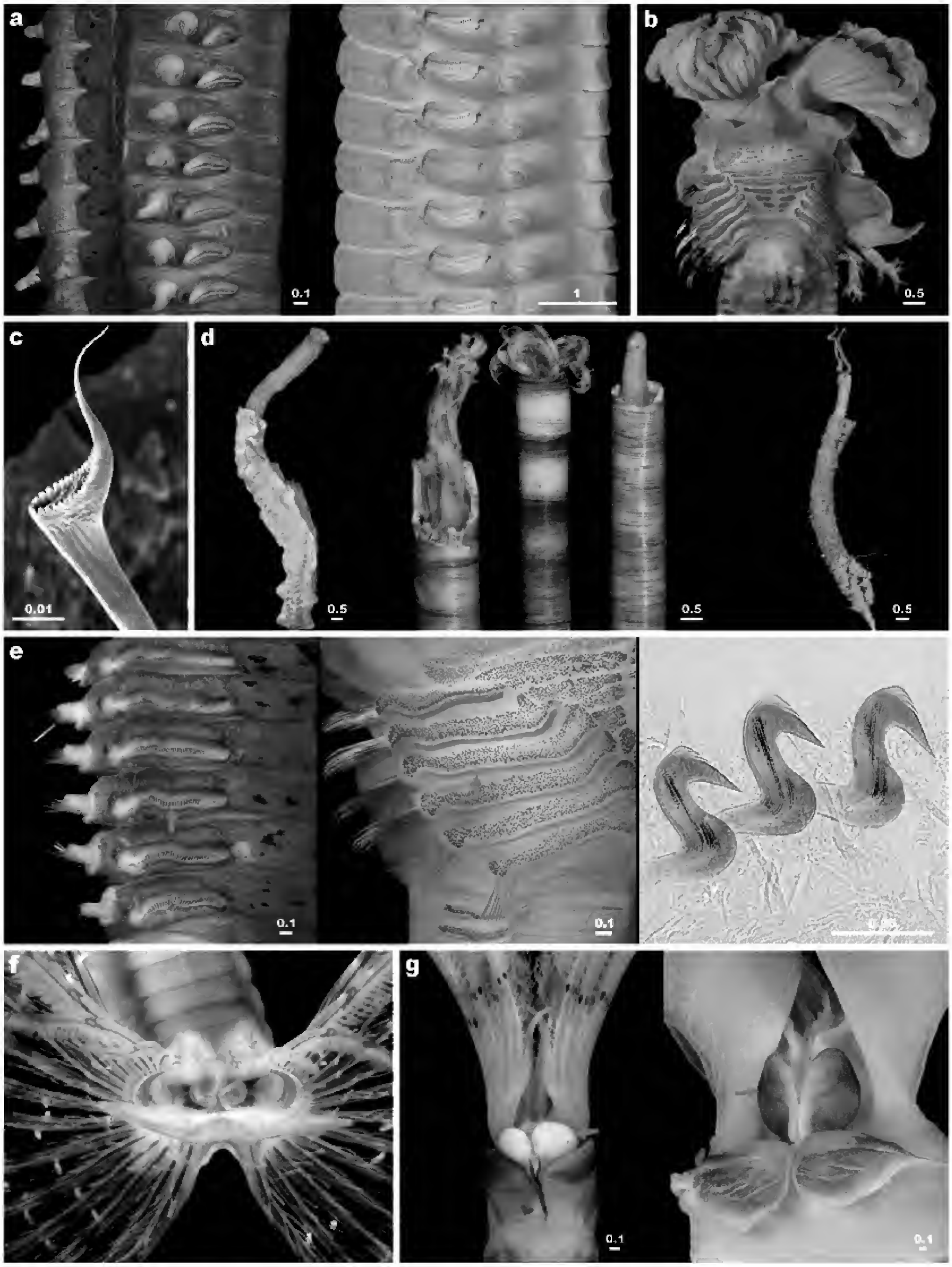

Figure 10. (a) Tori of *Branchiomma bairdi* and *Sabella spallanzanii* highlighted red. (b) Triangular depression in *Spirobranchus tetraceros* (stained with methylene blue) highlighted red. (c) SEM image of true trumpet-shaped chaetae of *Spirobranchus giganteus*. (d) Tubes of (left to right, respectively) *Spirobranchus taeniatus*, *Bispira serrata* and *Pseudopolydora paucibranchiata*: calcareous in Serpulidae (*S. taeniatus*) and muddy in Sabellidae (*B. serrata*) and Spionidae (*P. paucibranchiata*). (e) Uncini of (left to right, respectively) *Branchiomma bairdi*, *Spirobranchus cariniferus* (stained with methylene blue), and close-up in *Bispira manicata* (stained with methyl green). (f) Arrow indicates ventral lip in live specimen of *Branchiomma arctica* (photo: © Alexander Semenov). (g) Collar region of *Bispira serrata* and *Sabella spallanzanii*; arrows indicate ventral sacs. All scales in mm.

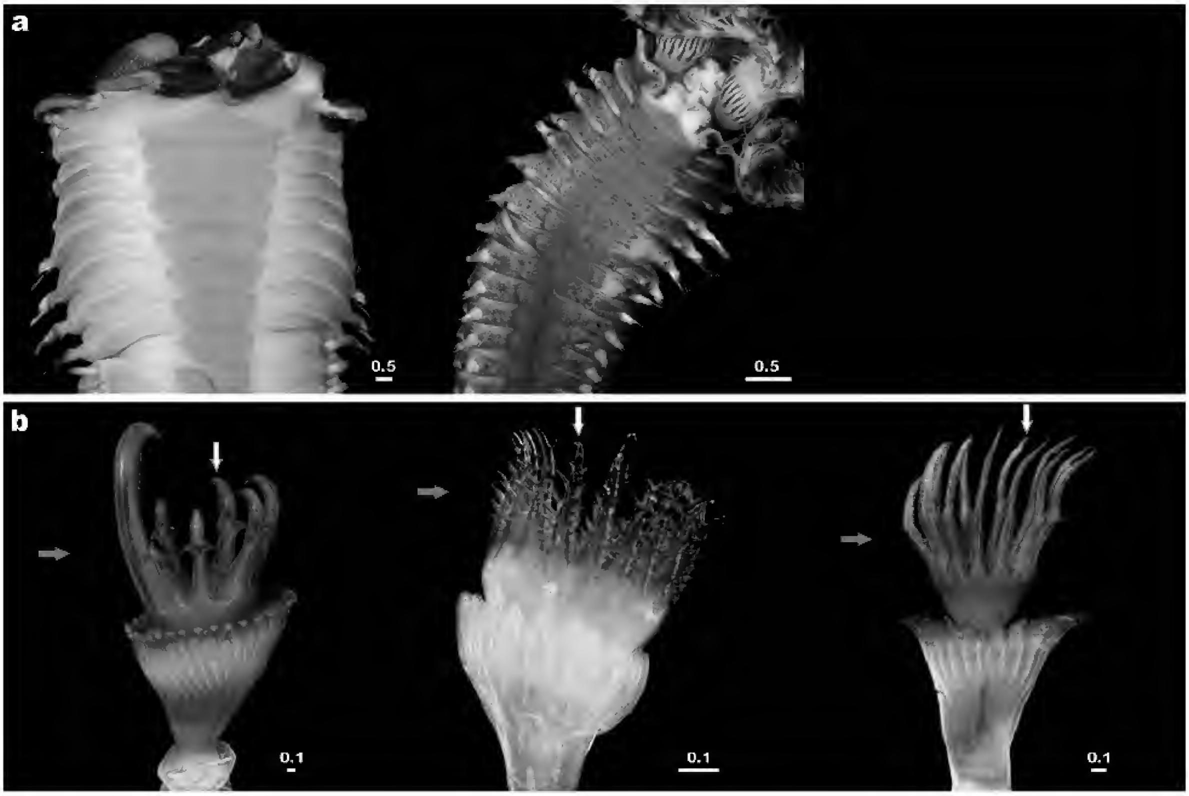

Figure 11. (a) Ventral shields of *Bispira porifera* (stained with methylene blue) and *Branchiomma bairdi* highlighted red. (b) Opercula of (left to right, respectively) *Hydroides heteroceros*, *H. longispinosus* and *H. sanctaecrucis*; red arrows indicate verticil; outlined arrows indicate verticil spines. All scales in mm.

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Bertil Åkesson (1928 – 2013) obituary

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Bertil Åkesson (photograph from a private source).

Bertil Åkesson passed away June 25, 2013 at the age of 85, mourned by his wife Birgitta and son Bengt with family.

Bertil was born in Lund and grew up at Skabersjö Castle, where his father Alfred Åkesson worked as estate trustee. After graduating in Malmö, Bertil started his academic career at Lund University in 1948. He took his master's degree in 1951 and his doctorate in 1958 in zoology on sipunculids. Bertil married Birgitta Stendahl in 1960, also a biologist working as a teacher trainer. Bertil had a position for 12 years as Associate Professor of Zoology in Lund, but in 1970 he transferred his personal research fellowship to the University of Gothenburg, where he got closer to the marine facilities on the Swedish west coast.

As the Department of Zoology in Gothenburg grew, the broad topic of structural and ecological zoology became unmanageable to be handled by a single professor. As a professor in Zoology, Bertil Åkesson then in 1986 took over the responsibility for the ecological activity at the Department. He was also Head of Department for two periods. He retired in 1993 but continued his research at the institution for many years, publishing what became his last paper in 2011. Bertil's long research career reflects the major changes in zoology during this epoch. Throughout the first part of his career he developed a great skill in comparative morphology and published three major studies on sipunculans. During the 1960s, he broadened his research field with embryology, mainly working with polychaetes, soon to become his central model and analysed with dedicated enthusiasm.

Bertil's influential pioneering work showed that a group of polychaete species (*Ophryotrocha*), with small body size, short generation time and resistance to a broad range of environmental conditions, was well suited for laboratory experiments. In these polychaetes, he saw great potential to analyse some of that time's central research problems, such as speciation, behaviour, mode of reproduction and life cycle strategies. Bertil's work established the group as a model organism for both basic evolutionary questions as well as an ecotoxicology model for the effects of marine pollutants. He held more than 20 species in culture in his lab, some of them continuously for 30–40 years, and he played a key role in distributing these species to laboratories all around the world.

Bertil Åkesson was well known in his field of research and he had broad international collaboration with marine research stations and universities in Europe as well as in the USA and Australia. His international contacts were beneficial to graduate students as well as younger colleagues in that he enthusiastically encouraged and arranged for their visits to foreign institutions. In Sweden Bertil contributed to the expansion of the marine field station at Tjärnö at University of Gothenburg, where he also supervised a number of PhD students. He was also active at the field station at Kristineberg, from where it is not far to Högby, where Bertil and his family have had their summer residence since 1966.

Bertil Åkesson's work at the department was dominated by research and, in the years closer to retirement, administration as Head of the Department. He was also a tutor and university teacher. In all, Bertil was factual, honest and impartial, efficient, positive, and supportive. He was gifted with much humour that helped to solve many knots, often with a merry laugh. We miss our dear colleague Bertil, his good humour, positive view on life, and irrepressible enthusiasm for science.

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M. Nechama Ben-Eliahu, 4 January 1935 – 23 March 2014. Obituary and some personal reminiscences

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M. Nechama Ben-Eliahu at the 10th International Polychaete Conference, Lecce, Italy, in June 2010 (photograph by Sergio I. Salazar Vallejo).

Nechama Ben-Eliahu was born in New York City in 1935. After a B.A. and M.A. at Indiana University she initially embarked on a teaching career. A lecture by David Ben-Gurion (Israel's prime minister at the time) that she attended in the early 1960s turned out to be a life-changing experience and she decided to emigrate to Israel. She arrived in Israel as a single mother with a young son on the 22nd of June 1962. Years later she would still celebrate that date as the most important day in the year, even more significant than her birthday. She initially settled in Haifa, first working as a teacher, but soon taking on a research position at the Sea Fisheries Research Station in Haifa, working on a major joint research project of the Smithsonian Institute and the Hebrew University of Jerusalem sorting invertebrate taxa from the Red Sea and the Mediterranean.

Ariel: When Nechama would talk about her work on serpulids, she often told the story of how she came to work on this obscure taxon. When she first joined the staff of the Sea Fisheries Research Station, she was told she could choose between echinoderms and polychaetes. She asked to look at some samples to decide. An average sample included no more than five species of echinoderms, which could all be easily identified to species. Such a sample included over 20 species of polychaetes, none of which could be identified easily. It was immediately obvious to her which she should pursue.

Harry: My first – at the time still infrequent – contacts with Nechama must go back to the early 1970s when Por's publication about Lessepsian migration in *Systematic Zoology* caught my attention, and Nechama started to publish on that topic too. I forgot the exact year, but she still lived in Beersheba. We mainly exchanged reprints, an occasional piece of information or check on identification of serpulids. My first brush with the "peoples' person" she was occurred during the 1st International Polychaete Conference in Sydney, 1983. Soon after that came the first of a couple of visits to the Netherlands, during which she generally was based at least some part in our home in Nieuwegein.

Soon after arriving in Israel, Nechama remarried, adopting her husband's two sons from a previous marriage as her own. She moved to the southern city of Beersheba, continuing her work on polychaetes, now as part of her Ph.D. thesis on the diversity of polychaete cryptofauna, under the joint supervision of Profs. Dov Por and Uriel Safriel from the Hebrew University. Her life was split between Haifa, Beersheba (where she also worked as a teaching assistant at the University of the Negev) and Jerusalem. After a few years in Beersheba, she moved to Jerusalem on her own, single once again, and lived in a flat in Jerusalem, where she hosted many guests and visitors over the years.

Harry: In 1990 Nechama invited me to her apartment for a six-week period in Israel, three weeks of fieldwork to sample the ongoing Lessepsian migration, three weeks of labwork. Jerusalem proved to be a small world. When we had dinner with friends of hers, I recognized the church on a painting as the one I was married in; her friend had lived in the town where I went to grammar school and had been a regular visitor to my wife's neighbours. When she introduced me to my prospective diving buddy, Shmuel Pisanty, I did not immediately recognize his face, but she was even more surprised than I when Shmuel and I realised that we had enjoyed each other's cooking in 1967, both doing research in the Zoological Station in Den Helder (nowadays NIOZ), the Netherlands. Of course Nechama showed me around in the Biblical Zoo in Jerusalem, of which she was a board member, where she proudly showed me her visualized idea: animal footprints in concrete to educate the younger generation. Nechama and I had long and sometimes heated discussions over material, interpretations, emerging texts, whatever. The result of the fieldwork and those discussions was published in 1992. On a later occasion in Nieuwegein, I heard her admit to my wife that she sometimes contradicted me just for the sake of the argument. She appreciated very much that I arranged a meeting with the director of the Royal Amsterdam Zoo, Artis Natura Magistra, whom I happened to know. That time, by the way, we again found out that it is a small world: visiting a mill-museum in Holland we stumbled across one of her political friends from Jerusalem!

Ariel: Nechama's life moved in many different circles, and she knew a huge number of people. There was the circle of zoologists (Polychaete lovers and others) – both in Israel and abroad – who all seemed to be her best friends. There was the circle of the American community in Jerusalem, where she was very active and apparently known by everyone. There was the circle of the Biblical Zoo, where she was an active board member. And, of course there was political activity. Nechama devoted almost every spare minute to politics. She was a regular demonstrator for peace, reconciliation and understanding with our Arab neighbors. She was constantly handing out flyers, standing at vigils or marching in protest. She became an active member of the liberal party Ratz, which later reformed to give rise to Meretz. Here also she was an active board member, knew all of the members of parliament and city council personally (and had all of their respect). She frequently said to me that she made a decision to devote a certain percentage of her time (I think she said 10%, but it was closer to 50%) to make this country a better place for her children and grandchildren. She lost a granddaughter to a terrorist attack, and this only made her reserve stronger to continue acting for Peace.

Harry: Amongst others we shared a passion for politics, she to the far left, we (my wife and I) to the right of the middle (we have a lot of political parties in the Netherlands, even more than in Israel). Being an active member of the "Peace Now" movement, she regularly demonstrated against the settlement policy and for solidarity between all inhabitants of Israel. She told us that she often had been abused by pedestrians during those demonstrations. Nevertheless she persisted in these efforts even after her 17 year old granddaughter, whom we had visited together in 1990, was killed in a Hamas suicide attack in 2003. Another common trait was our love for animals, hers for cats, mine for dogs. I vividly remember the devastation in her face when, in our living room, she was informed by phone that her cat in Jerusalem had disappeared.

Upon completing her Ph.D., Nechama remained at the Hebrew University of Jerusalem, as the curator of aquatic invertebrates in the National Natural History Collection. Nechama's public and scientific activity earned her many accolades. She was chosen as an honorary citizen of Jerusalem for her work in the Zoo and other public activity. She was also made an honorary member of the Zoological Society of Israel for her work on Lessepsian migrants.

Ariel: I first met Nechama as a graduate student. I had no interest in serpulids or any other worms at the time (my work was on amphibian development). Even though I knew nothing of her work, she had a very clear presence in the department. Her general liveliness and optimism were extremely contagious, and she was well-liked by everyone. She was the "older sister" of many of the graduate students in the department, and volunteered to do English editing for everyone's papers and presentations.

Harry: But of course we often talked shop, more and more calcareous tubeworms, though she kept an interest in other polychaetes as well. In 1986 she came to Amsterdam with an outline of a manuscript on some extremely small worms she had from the Red Sea and Cyprus, which she believed to be new to science and wanted to give the Arabic name *Hadiya* (gift). However, I had the feeling having seen a description (and drawing) of an operculum very similar, if not the same, in papers by Bush and Straughan, and advised her to have a good look at those papers. A year later I was sent a manuscript for review by a well-known journal. It was the same manuscript, finished but without what I thought were essential modifications. She dearly wanted to make the statement included in *Hadiya*. I phoned the editor and told him that I hardly could remain anonymous, since I could only give the same comments as a year before. He asked me to review the paper anyhow, so I bought a different daisy wheel for the printer (she would recognize my lettering from my frequent letters), asked my colleague to write some remarks in the margin of the manuscript, and sent off an "anonymous" review. After a couple of months Nechama sent me a letter asking my advice on two reviews she had received, one by Helmut Zibrowius and one a very long set of comments by an anonymous reviewer, almost as long as the original manuscript. I could only confess that I had been that anonymous reviewer, and she immediately asked me to come in as second author. Once interested, she could be very tenacious, and in this case even went to the USA again to find new material in Bush's dry coral collections. Mind you, the tubes are hair thin and the dry opercula slightly over 0.1 mm wide! The rest you can read in our 1989 publication.

Ariel: When I returned from my post-doc to a faculty position at the Hebrew University, I was made academic curator of the aquatic invertebrate collection – the same collection that Nechama was the (now retired) curator of. Not having a background in natural history collections, I was greatly helped into the new position by Nechama and her great experience and passion for natural history. We became very close in the last few years, talking almost daily, often over lunch. We talked about a wide range of subjects, as wide as Nechama's interests, from science and zoology, through university politics and, of course, frequently about national politics. I heard many stories from her about the Golden Years of the Collections; her field work in the Suez Canal weeks after the end of the 1967 war; the great collecting expedition in the Western Mediterranean on board the *Meteor*; wading in boots in the mud near Haifa Harbor; her trips around the world to the International Polychaete Conferences (I think she attended every one of them, except for the last one in Sydney). The last meeting she went to was the one in Lecce. She brought me the conference T-shirt as a gift, and I still wear it and think of her.

Harry: I mentioned her capacity for tenaciousness. My preferred animals are big and showy, or with nice and very distinctive opercula thought to be clear-cut species, such as the X-mas tree worms *Spirobranchus* and *Hydroides*. She nevertheless lured me into small to very small worms three times, with *Rhodopsis* (above), with the mistakes made in juvenile *Hydroides* (published 2005), and the taxonomic mess of *Salmacina*. Admittedly, we have not solved the last problematic taxon, one would need DNA for that, but found at least some useful characters, which I until then had thought impossible. Her Magnum Opus (with some help from my side) of course was *Serpulidae (Annelida: Polychaeta) from the Suez Canal—From a Lessepsian Migration Perspective* (*a Monograph*), 2011. Her drive made us even search for additional data for this reconstruction in time – of serpulid settlement in the Suez Canal – in dry tubes attached to molluscs in Naturalis, painstaking work which in Dutch would be called "monks' work"! With her impulsive continuous improvements, even when I was in the middle of editing parts, she made me mad and almost drove me crazy, but finally a lot of work came together in this paper.

Ariel: About 4 years ago she went into hospital for what supposed to be a routine operation, during which it was discovered she had cancer. A series of medical errors and complications led to the fact that for the last few years of her life she was in and out of hospitals. She didn't lose her optimism or her energy. She continued to come to work whenever she could (despite being retired for over 10 years). She would always apologize if she missed a day's work because of a doctor's appointment or because she wasn't feeling well. I had to argue with her to convince her that it perfectly legitimate not to come to work under these conditions, and we are all happy to see her, but nobody would be angry with her if she didn't come in. She made the last edits to her monograph and went over the final proofs, while in the hospital, much to the surprise (and respect) of the hospital staff. As a sign of gratitude, she invited the entire staff of the ward she was in to a guided visit to the Zoo.

Harry: The last years, especially after she had been diagnosed with cancer, we spoke (and sometimes saw) each other almost every two weeks by phone (or on Skype). Even then she remained a "peoples' person", worrying about others. In hospital November 2011 she called to inform me that her email account had been hacked and misused (which I had noticed an hour before) to solicit money from her contacts. She asked me to warn the annelid community. Unfortunately the begging proved to be successful with one of her local acquaintances. Nechama always was very reticent to talk about herself, notwithstanding the fact that she was perfectly aware that both my wife and I know cancer first hand, and as a straightforward Dutchman I can ask very direct questions. She always enquired about our health, and that of the children/grandchildren, and indeed kept us informed on the (family) situation in Israel. Summer 2013 she decided not to go to the 11th International Polychaete Conference in Sydney, she had not missed a single one before, sent me the presentation of her intended talk, but nevertheless told me she hoped to go to the next conference in three years time. Typical for her state of mind. After that her scientific communications dwindled to nil, although she tried, the effort proved to be too strenuous. However, even a few weeks before the end she still showed a student, interested in *Spirobranchus* and with whom I was exchanging information, specimens present in the Hebrew University Collections. When she was not on Skype any longer, nor answering mails, I knew that her situation was bad. Some 10 days before she died I last heard her voice over the phone, weak as the way she felt. Amongst my colleagues, Nechama ranked as a friend. I will miss her.

Ariel: In 2012 Nechama and I got a joint grant to look at Mediterranean serpulids, repeating the survey done by her and Harry 20 years earlier. Work on this project filled the last years of her life. She was not able to do sampling herself, so we hired a professional marine biologist to sample for us. I went with her to meet him on the Kibbutz where he lives on the Mediterranean coast, and she taught him how and where to sample. She waded into the water (frail as she was), and pulled specimens out with her hands. I think this was the last time she was "in the field". About a week before her death a sample of serpulids arrived for her to go over. Sadly, she never had a chance to see them.

Nechama Ben Eliahu passed away late in the evening of the 23rd of March 2014. Her funeral was attended by several hundred people. The eulogies lasted for over 45 minutes (very unusual for Jewish funerals), with acquaintances from all her different circles speaking in her memory. They all praised her kindness, her wonderful cheerfulness and her total dedication to everything she did. She will be sorely missed by all.

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Michel Bhaud (1940 – 2012) obituary

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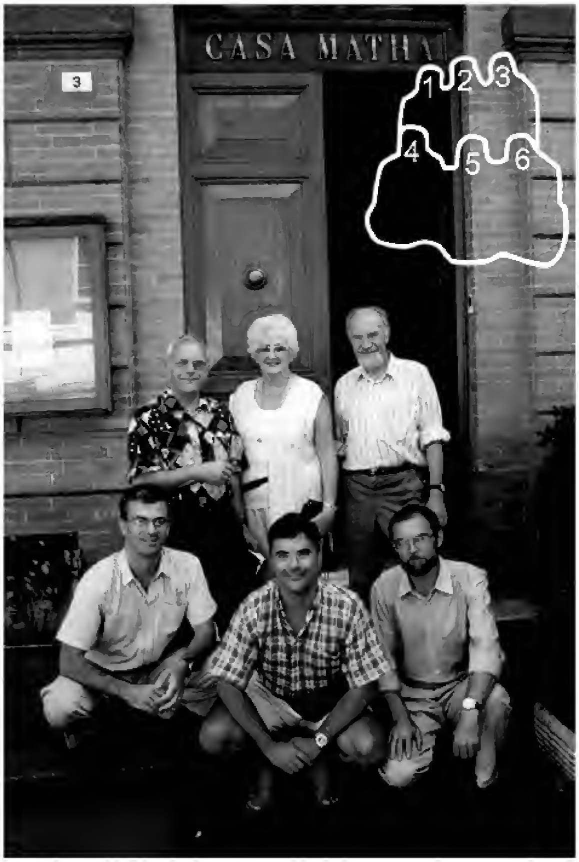
It was with great sadness that we learned of the death of Professor Michael Bhaud on May 8 2012, when he was only 72 years old.

Michel Bhaud was born the 30th of July 1940 in Vebret, a small village shadowed by the basaltic plateau of Chastel-Marlhac, in Cantal (Auvergne, France). He studied in Caen, obtained a DEA in Biological Oceanography in Paris (1964), and became Doctor in Natural Sciences in 1971. His Doctoral Thesis was directed by Professor Pierre Drach, having as a member of the jury a Professor already well-known as a polychaetologist: Lucien Laubier.

Michel entered the CNRS in October 1966 as "Stagiaire de Recherche" and soon moved to "Attaché de Recherché" (1968) and "Chargé de Recherché" (1973). He was then Maître-Assistant at the University of Paris VI (1973-1976) and finally became "Maître de Conference" since 1980. In 1975 he received the bronze medal of the CNRS.

Michel was also lucky with his military service, which he was able to undertake within the framework of a technical cooperation in the ORSTOM Oceanographic Centre at Nosy-Be (Madagascar), from February 1967 to May 1968. His time spent at Nosy-Be, as was always the case with the various challenges and opportunities he faced, resulted in scientific papers, in this case an important series of papers on larval biology (e.g. Bhaud, 1969a, Bhaud, 1969b, Bhaud, 1972b).

Being as active in teaching activities as in research, Michel participated in all levels of student training, including practical lessons during the annual visits to the OOB by the 3rd cycle students of the University of Paris, in addition to the supervision of numerous pre- and post-doctoral students, all of whom successfully gained their theses and proceeded to post-doc projects. Many now occupy senior positions in laboratories in France and around Europe. All (Daniel Martin included) were infected by his love of nature, its creatures, and the science that tries to describe them. His enthusiasm was contagious and he has been a guide for their studies from the moment their collaborations began during their 'stages' in the



Meeting of the INTAS project on the sibling species problem held in Ravenna (1999): 1. Michel Bhaud; 2. Phyllis Knight-Jones; 2. Vladislav Khlevovitch; 4. Marco Abbiati; 5. Daniel Martin; 6. Temir Britayev (Absent: Alexander Rzhavsky).

OOB, where all frequently enjoyed the interesting conversations at the social and scientific parties that Michel kindly offered at his beautiful house in Mas Parer, a small village half way from Banyuls-sur-mer to the Col de Banyuls, half way from the sea to the Pyrenees.

Michel has been an important mentor, a good friend, an exemplary person, and an excellent scientist influencing for the better all who worked with him. To typify these experiences, following Daniel Martin's first 'stage' in Banyuls in 1994, they established a continuous collaboration that resulted in a long-lasting friendship, but also in the publication of scientific papers on several of Michel's preferred topics, from larval biology and recruitment to the life cycle of *Eupolymnia*

nebulosa and the taxonomy and ecology of Chaetopteridae and Oweniidae (Arnoux et al., 1995, Bhaud et al., 1995a, Bhaud et al., 2006, Bhaud et al., 2003, Cha et al., 1997, Martin et al., 1996, Martin et al., 1998, Martin et al., 2008, Martin et al., 2006, Martin et al., 2000). They also collaborated in several joint projects. Among them, a French/Spanish INTEREG project and an INTAS project on the sibling species problem deserve special mention. The international reputation and prestige of Michel resulted in ongoing collaboration between DM and other well-recognised scientists working on marine ecology and polychaetes; these included Vladislav Khlebovich, Marco Abbiati, Phyllis Knight-Jones and Temir Britayev (Fig. 1). No doubt many others would be able to recount similar influences, resulting in new insights and new research directions typified by Daniel Martin's current interest in the *Haplosyllis spongicola* complex.

Michel's vast experience and knowledge enabled him to play an active role in the governance, planning and supervision of National and International research programmes. His role in the French National Research Program on the Determinism of the Recruitment (PNDR) has to be highlighted in particular; this was led by Michel for several years and gave rise to a series of internationally important studies on recruitment and larval biology and ecology that were revolutionary and will always remain a matter of discussion.

Michel's work at the Laboratoire Arago of the OOB, began at the time when Professor Drach was appointed Director (1965 à 1976). Soon he joined the Plankton Team, where he was in charge of the study of the polychaete larvae (always encouraged by Lucien Laubier). According to his own words: "Malgré les travaux de THORSON, tout reste à faire en Méditerranée. Alors commence le lent travail d'identification par élevages après la pêche en mer où ma présence est constante." Since then, he intensively worked on the study of polychaete larvae, taking many different approaches: taxonomic identifications, faunistics, dispersal, biogeography, settlement, feeding modes, swimming behaviour, and so on (e.g. Arnoux et al., 1995, Bhaud, 1966a, Bhaud, 1966b, Bhaud, 1967a, Bhaud, 1972c, Bhaud, 1974d, Bhaud, 1983d, Bhaud, 1986, Bhaud, 1988c, Bhaud, 1989, Bhaud, 1990a, Bhaud, 1991, Bhaud, 1993b, Bhaud, 1998c, Bhaud, 2003, Bhaud & Cazaux, 1982, Bhaud & Cazaux, 1987, Bhaud & Cazaux, 1990, Bhaud et al., 1990, Bhaud et al., 1994a, Bhaud et al., 1995a, Bhaud et al., 1995b, Bhaud & Duchêne, 1989, Bhaud & Duchêne, 1996, Bhaud & Fernandez-Alamo, 2000, Bhaud & Grémare, 1988, Bhaud et al., 1999, Bhaud & Fernández-Alamo, 2001, Duchêne et al., 1992, Marcano & Bhaud, 1995, Nozais et al., 1997).

After several years focusing on plankton, Michel started to enlarge his scientific interests to the benthic domain, studying the energetic links between ecosystems and the energy flows at the individual level, which at that time corresponded to a particular component of the studies conducted by the research group on "Structure and Functioning of the Benthic Ecosystem" at the Laboratory Arago. Accordingly, Michel argued that "Cette modification progressive de l'orientation' des recherches permet, en dépassant les questions liées à une unité zoologique particulière, un regroupement des différents sujets autour d'un thème lié au fonctionnement des écosystèmes et à leurs relations." (e.g. Bhaud, 1988b, Bhaud et al., 1995b, Cha et al., 1997, Martin et al., 1998, Martin et al., 2000).

The focus on benthos and the combined interest on the factors affecting larval dispersal and the distribution of the adult populations also led Michel to pay attention to taxonomic problems and so he became one of the best representatives of a remarkable generation of French polychaete taxonomists. For instance, his work on Chaetopteridae (e.g. Bhaud, 1966a, Bhaud, 1969d, Bhaud, 1969f, Bhaud, 1972a, Bhaud, 1975e, Bhaud, 1977b, Bhaud, 1978, Bhaud, 1998b, Bhaud, 2003, Bhaud, 2005, Bhaud & Fernandez-Alamo, 2000, Bhaud et al., 2002a, Bhaud et al., 2006, Bhaud et al., 1994c, Bhaud et al., 2003, Bhaud & Petti, 2001, Bhaud et al., 2002b, Martin et al., 2008, Nishi & Bhaud, 2000, Nishi et al., 2004b, Nishi et al., 1999) and Oweniidae (e.g. Koh & Bhaud, 2001, Koh & Bhaud, 2003, Koh et al., 2003, Martin et al., 2006, Pinedo et al., 2000) are still matter of reference.

In addition to the more than 120 scientific papers he wrote during his prolific scientific life (see a complete reference list at the end of this text), Michel was very active in many other aspects of scientific life. For instance, since the first one in Sydney, where he presented a paper on the oocytes of *Sabellaria alveolata* (Bhaud et al., 1984), his presence and talks in the different Polychaete Conferences (and also his participation in social events) will always be within the best souvenirs of most of us (Figure 2). He also participated in and presented papers at many of the meetings of the International Society for Invertebrate Reproduction for instance those held in UK, France (Lille), Japan, and Dublin and at international meetings relating to aquaculture and fisheries. In this way he ensured that polychaete research was well represented at important scientific meetings with a broader taxonomic remit. As global concerns about climate change began to emerge, he soon realised the significance of his work on polychaete reproduction and larval development in this important context and took part in international meetings to consider what the ecological consequences of climate change might be (Bhaud et al 1994b). It was characteristic of Michel, throughout his publishing career, that he thought deeply about the wider significance of his work and many of his papers are fine examples of scientific philosophy and thought.

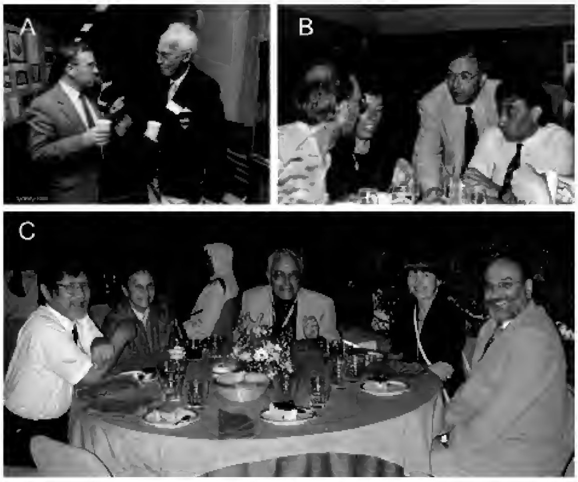
It is testimony to the character of Michel that, from the many international collaborations that characterised his long and active career, grew many personal friendships as both authors can testify. As a polychaete biologist, Peter Olive shared many of the Michel's interests especially those in different aspects of reproductive biology. Michel's publications on the timing of polychaete larval appearance in different biogeographical regions (e.g. Bhaud 1972c, 1988, 1991, 1998; Bhaud and Duchêne 1989, 1996; Bhaud et al 1990, 1992, 1995; Bhaud and Fernández-Álamo, 2000) strongly influenced much of the work done at Newcastle University and served as a key starting point for taught courses on reproduction and seasonality for honours Zoology and Marine Biology students at Newcastle University. The mechanisms by which polychaetes, and other marine organisms, transduce environmental signals, the so called *proximate factors* determining the timing of breeding and the *ultimate factors*

through which an adaptive advantage is gained, has long interested marine biologists and the work of Michel is central to this problem. Michel understood the important distinction between these two aspects and, with his students and international collaborators, he contributed extensively to this important field of study, requiring, as it does skills in both field ecology and experimental, laboratory based, investigation (Bhaud 1982a,b,c; Bhaud and Cha, 1992; Bhaud et al 1994b; Cha et al, 1997; Martin et al. 1998). These studies will continue to inform current interest in the molecular biology of environmental signal transduction and biorhythmicity in marine organisms and continue to have importance in relation to climate change.

Michel was, throughout his research career, a natural collaborator, forging links around the world. He became involved with international training programmes and contributed much to the development of a new generation of researchers who would share his enthusiasm and who benefited from his unmatched experience and knowledge of polychaete larval biology. As an example, he participated as a lecturer in the first advanced polychaete training workshop organised by Maria Gambi at the Stazione Zoologica di Napoli's ecology laboratory on the Island of Ischia. Here, experienced teachers and researchers were joined by the vanguard of younger scientists working at the cutting edge of the latest taxonomic procedures to teach an international group of polychaete researchers at early stages in their careers. Michel's enthusiastic teaching in this milieu will have done much to cement the interest of those who attended many of whom are now among todays leading polychaete biologists and taxonomists.

Michel's involvement in research, graduate student development and training, and international scientific enterprises stimulated cross national collaboration in many different ways. He encouraged his overseas collaborators to become involved in the examination of PhD theses in the French way, and several of his overseas collaborators were invited to participate at a 'soutenance'. This was sometimes a linguistic challenge for English speaking members (Peter Olive included), but was always a pleasure and an honour. Most importantly it led to opportunities to discuss areas of mutual scientific interest and to forge further research collaborations. He also encouraged participation of overseas observers in the governance of those French National research programmes in which he was involved. Through this an avenue was opened for rapid the exchange of ideas between national programmes in different countries not otherwise formally linked. As an example, links developed between the CNRS PNDR programme alluded to above, and the UK NERC programme on Developmental Ecology of Marine Animals (DEMA) (see Atkinson and Thorndyke eds., 2001) being supported at the same time.

It was inevitable given the generous nature of Michel, that these collaborations lead to close personal friendships, and the authors of this appreciation make no apology for drawing on their own personal experiences in the knowledge that similar experiences will have been enjoyed by many others.



Michel Bhaud: A. with Wynn Knight-Jones at the 1st Polychaete conference in Sydney. B. at the 3rd Polychaete Conference in Long Beach. C. with Tomoyuki Miura, María Ana Fernándz-Álamo, Madamme Dauvin and Jean-Claude Dauvin at the 8th Polychaete Conference in Madrid.

Michel and his wife Yvonne, also a CNRS researcher at the OOB, had a daughter, Katy, and a son, Manu, and four grandchildren. An international exchange of teenage children between Michel's family and Peter Olive (to learn the language) as planned and instigated by Michel, was followed by family vacations at Banyuls-sur-mer for some 13 years. The hospitality extended by Michel and Yvonne, frequently involved an invitation to stay in their delightful house and always generously hospitality on the terrace of Mas Parer. Those memories of good companionship on the terrace overlooking the valley remain priceless and the friendships that developed between the young people at Banyuls and the visitors from England have endured to this day and are an unseen tribute to the generosity of spirit of Michel, his wife Yvonne and their children.

After a long and productive scientific life in which Michel always remained an important scientific guide and mentor as well as a kind and supportive person and friend, he retired in 2005, when he left the OOB. Sometime later, he courageously underwent a triple bypass operation, after which Michel lived at his beloved Massif Central, always devoted to his family and, particularly, to his grandchildren but also characteristically involved in local affairs.

Michel Bhaud has been one of the most important influences in our scientific careers and we feel that his abiding footprint will long remain with the entire polychaete community. For those who knew him at a personal level, his kindness and friendship will equally last forever. In presenting this appreciation of his scientific career we also hope to express our sympathy for his family, and for friends who survive him.

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Professor R.B. (Bob) Clark (1923 – 2013) – polychaete biologist and environmentalist: a pioneer in comparative endocrinology of reproduction, growth and regeneration.

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Bob Clark during a visit to China in the 1990s (photograph by Prof. Wu Boa Ling).

Professor R.B. (Bob) Clark, an outstanding scholar and leading polychaete biologist of his generation, died quietly at his home on 28th September 2013, shortly before his 90th birthday. He was born in London in November 1923, the son of Joseph L. Clark and Dorothy (nee Halden). In his later career he was widely acclaimed for his work in Marine Pollution, a field of study that he did much to establish and one that has ever increasing global significance [1]. He was appointed a member of the Royal Commission on Environmental Pollution[2] which, together with his authorship of the various editions of his text book on Marine Pollution (Clark, 1986-2001) and other major publications (e.g. Clark, 1982) rightly establish his pre-eminence in this field. He was also invited to give evidence at the Royal Commission Enquiry on oil drilling on the Great Barrier Barrier Reef which was chaired by Prof J.E. Smith FRS, then the Director of the Plymouth Marine Laboratory in the early 1970s. This enquiry was instrumental in defining the boundaries of the GBR Marine Park which was declared in 1975 and ensured that no oil drilling could occur within the park. This Park was later declared a World Heritage Area.

Bob Clark took a first degree in physics and graduated from Chelsea Polytechnic in 1944. After working for a while in this role he entered Exeter University to read Zoology in 1947, graduating in 1950. His first appointment as a zoologist was at the University of Glasgow and, while at the University of Glasgow, he was able to study in the USA at Universities of Washington, Seattle and University of California (Berkeley) where he was Assistant Professor during a Sabbatical awarded to polychaete biologist Ralph Smith. He also worked extensively at the Friday Harbor Marine Laboratory, teaching there till 1978. He was appointed to the University of Bristol as Lecturer in 1956, awarded a DSc by the University of London in 1965 and appointed to the positions of Professor and Head of the Department of Zoology and Director of the Dove Marine Laboratory at Newcastle University, UK in 1965. He was also honoured with election as a Fellow of the Royal Society of Edinburgh in 1970. Bob married Mary Clark, a USA citizen and they had a productive professional collaboration for many years. After his move to Newcastle upon Tyne, Mary returned to the USA to pursue an independent academic career and they divorced. Bob remarried and is survived by Sue, their two children, Juliet and Stephen and grandson Gus.

1 *From mimeos to e-copy – a tribute to Professor RB (Bob) Clark, founding editor of the Marine Pollution Bulletin. Marine Pollution Bulletin* 46(9):1051-1054

2 *8th report of Royal Commission on Environmental Pollution : Oil Pollution in the Sea* 1981 HMSO, London

Bob did much to establish polychaetes as models in experimental and evolutionary zoology and created vibrant, polychaete research schools at Bristol and Newcastle Universities, supervising and mentoring many of the next generation of polychaete scientists. He was also the supervisor in Bristol of Barrie Jamieson, who became a leader in the field of oligochaete biology (see Brinkhurst and Jamieson 1971, Jamieson 1971 supplementary bibliography). The relationship between the polychaetes and oligochaetes (clitellate annelids) was among the issues Bob addressed (Clark, 1969, 1978) that is finally being clarified with the aid of molecular data. As a supervisor, Bob generously encouraged his students to publish independently and did not follow the modern tradition of publishing jointly. His influence therefore extends well beyond his own publications. Athough it is not appropriate here to cite all the publications of his students, a supplementary bibliography listing some of the work arising from studies carried out under his supervision is included.

The polychaete studies begun at Glasgow University resulted in the publication of one of the first keys to Polychaete families in English (Clark, 1960a) and tellingly, a short paper on pelagic swarming in Scalibregmatidae (Clark, 1954), a topic which would re-emerge in various forms during much of his research career. His ecological studies include a survey of the distribution of *Nephtys* species in the British Isles (Clark, Alder and McIntyre, 1962; Clark and Haderlie, 1960) and in California (Clark and Haderlie, 1962) and the food of *Nephtys* (Clark, 1962). The problem of niche partitioning and geographical distribution of *Nephtys* species he addressed, has been reprised by former students (Olive and Morgan, 1991, supplementary bibliography) and remains a topic of importance for the polychaete community.

Bob's early training, and initial career in mathematics and physical sciences, underpinned much of his work on polychaetes, especially his investigations into biomechanics and movement and his work in this field influenced many others. He became an expert histologist and combined studies of polychaete structure, often in collaboration with Mary Clark, with experimental studies (Clark, 1956, 1958, 1962; Clark and Clark, 1960a, b). He re-appraised the nature of undulatory swimming in polychaetes, demonstrating that, in species with prominent parapodia, the wave form propagates from tail to head (direct locomotory wave) and the power stroke of the parapodium, exerted at the crest of the wave, produces the main propulsive force (Clark, 1964; Clark, 1976, Clark and Tritton, 1970). He also investigated swimming in smooth bodied species, such as Opheliidae, in which the parapodia play no significant role in the generation of locomotory forces and the locomotory wave is retrograde as in other smooth bodied worms and fish (Clark and Hermans, 1976). Colin Hermans became a close friend of Bob's and has provided the authors with illuminating reminiscences of his early scientific career and influence in the States. His investigations into the role of the coelom and septa in the shape changes, that enable annelids to work in their environment, was fundamental to his thinking and his studies extended to investigations of non-coelomate invertebrates (Clark and Cowey, 1958). The combination of his histological and experimental investigations, together with his rigorous scholarship, enabled him to produce a definitive review of the evolution of swimming forms among annelids in relation to reproduction (the phenomenon of epitoky) (Clark, 1961) which remains a classic and is an essential introduction to the subject. As a student of biomechanics in annelids he became greatly interested in the deeper evolutionary implications of the relationship between form, function and phylogeny of metazoans. His interests culminated in a major synthesis, the book "The Dynamics of Metazoan Evolution" (Clark, 1964), which remains a masterpiece of scholarship and is a testament to the depth of his knowledge and thought.

'The Dynamics' subtitled "The origin of the coelom and segments" addressed metazoan phylogeny, a topic which has re-emerged in current biology in the wake of the genomic revolution, and to which the polychaete community is continuing to make important contributions. In it he, addressed theories relating to metazoan relationships in the light of "the principles of comparative morphology", which he argued, "must be taken into account when phylogenies are proposed, but which have hitherto escaped serious discussion in this context". In particular he explained, "as a student of the annelids, he was exercised by two outstanding problems: the nature and origin of the coelom and of metameric segmentation". He proceeded to a detailed analysis of the relationship between structure and movement in various grades of metazoan organisation, adopting what he described as, " ...a purely functional point of view". He then proceeded to a discussion of various theories relating to possible phylogenies of Metazoa in the light of comparative morphology and biomechanics. He recognised that "it is certain that many of the derivations of major groups of organisms that are accepted now will be re-examined, rejected and changed in the future." But, he cogently argued, "stem forms, from which several modern phyla diverge, must be possible animals...they must be conceived as living organisms, obeying the same principles that we have discovered in existing animals, and any new structures... must have conferred some selective advantage upon them". The argument that any putative ancestral, or primitive organism, must have obeyed the same physical laws as living organisms, is as valid now, in the 'molecular age', as it was then. Bob continued to evaluate theories relating to the relationships between worm like phyla (Clark, 1969, 1978). This was, of course, well before molecular and phylogenetic studies of polychaetes which are now providing new insights into these problems and which, a perhaps rather incredulous Bob, discussed with Peter during their morning chats over a coffee and a whisky in his later years.

To return to Bob's development as a polychaete biologist, during his early years at Glasgow, when he held a post as Assistant Professor at the University of California (Berkeley), and worked at the Universities of Washington and Seattle and Friday Harbor Marine Laboratory he formed many deep friendships and developed a deep interest in the emerging fields of neurosecretion and comparative endocrinology of invertebrates. He subsequently made a major impact on the comparative endocrinology of growth, regeneration and reproduction in polychaetes that indirectly led to subsequent endeavous to breed worms commercially and to the

establishment of aquaculture businesses in Europe, China and SE Asia (see Olive, 1999, supplementary bibliography).

Invertebrate comparative endocrinology, was at the time dominated by studies of insects and Bob, together with contemporaries, Hauenschild in Germany and Durchon in France, stimulated an interest in the endocrinology of polychaetes. His studies of the histology of the *Nephtys* brain (Clark, 1955, 1958a,b,c), laid the foundations for experimental studies of *Nepthys* endocrinology as subsequently pursued at Newcastle University by Peter Olive and his students and, for the masterful ultrastructural studies of his former student D.W. Golding, which lead for instance to the discovery of 'neurosecretory endfeet systems' with ramifications for neurosecretory studies throughout the metazoa.

While at the University of Bristol (UK) his research largely focussed on growth, regeneration and the 'once per lifetime' switch to reproductive maturation, typically observed in Nereididae but he also reviewed the subject in great depth providing the key reviews for anyone seeking an entrée into the subject (see Clark, 1965; Clark and Olive, 1973). His chosen model organism for his own studies was the estuarine *Nereis diversicolor* (now *Hediste diversicolor*), a species in which epitoky has been suppressed, but in which sexual maturation and the ability to regenerate lost caudal segments are, nevertheless, negatively linked to the onset of sexual maturation. This complemented the work of the Durchon laboratory at Lille, France, on the epitokous species *Perinereis,* and that of the Hauenschild laboratory in Germany, using *Platynereis dumerili* as the experimental model. The nature of the regulatory control by the supraoesophageal ganglion proved difficult to establish (e.g Clark and Bonney, 1960, Clark and Ruston, 1963) but Bob's student at Bristol, D.W. Golding, brilliantly established the permissive nature of this control and demonstrated the existence of a caudal growth field, such that the number of segments regenerated is a function of the number lost and not, as previously thought, determined by the level of hormonal output (Golding, 1967e-f, supplementary bibliography). The proof that the ganglion has a permissive role, essential for segment proliferation, but not itself determining the rate nor the number of segments regenerated, is crucial to our understanding of caudal regeneration in nereidids and given the resurgence of interest in segment formation in polychaetes (especially in nereidids) following the discovery of pan-metazoan Hox-gene regulatory system, this work remains highly relevant and will no doubt lead to further investigations of the nature of the influence of the so called juvenile brain hormonal function in nereidid regeneration. His studies of *Hediste diversicolor* also included aspects of behaviour and learning (Clark, 1960 b, c, d), and his postgraduate student Stuart Evans also took this subject forwards (Evans, 1969, supplementary bibliography).

Following his appointment at Newcastle University, Bob re-established a polychaete based research school, both on the University Campus and at the Dove Marine Laboratory. He attracted a large number of visiting scientists from USA and around the world to work at the Dove Marine Laboratory including Marianne Pettibone, Ralph Smith, Larry Ogglesby, Colin Hermans, Fu Chiang Chia, Arthur Fontaine, Bao Lin Wu and Son Lin Zhang, making the Dove Marine Laboratory, a dynamic research environment for a new generation of polychaete students. These included the authors of this appraisal (PJWO and PAH) but also John Daly, Peter Gibson, Peter Garwood, Ivan Estcourt, Evelyn Jaros and others (see selected publications of their student work in the supplementary bibliography). Students at this time were encouraged to explore the diversity of polychaete life histories, with a particular focus on comparative aspects of reproduction and the timing of reproduction. He encouraged an experimental approach and tellingly distinguished clearly between the so called 'ultimate' and 'proximate factors' controlling the timing of reproductive events (Clark, 1979). He gave his students a free head in the choice of experimental material and this resulted in the study of a remarkable array of polychaetes, drawn from several different clades. The species studied at this time included: *Cirratulus cirratus* (Cirratulidae, with Peter Olive), *Dodecaceria* spp. (Cirratulidae, with Peter Gibson - see Gibson and Clark, 1976), *Melinna cristata* (Ampharetidae, now *Melinna elisabethae*, with Pat Hutchings), *Harmothoe imbricata* (Polynoidae, with John Daly, Peter Garwood), *Fabricia sabella* (Sabellidae, Fabriciinae with Dave Lewis, Evelyn Jaros). This approach continued to be a feature of the Newcastle polychaete school and over the next few years, the array of species studied was expanded to include members of the Nephtyidae, Phyllodocidae, Hesionidae, Spionidae, Capitellidae and Arenicolidae, leading to an appreciation of the diverse patterns of control of reproduction in polychaetes commensurate with their long evolutionary history (Clark, 1979; Clark and Olive, 1973). As a supervisor Bob had a 'hands off' approach, so each student had to solve the problems they encountered in their own way, sometimes made all the more difficult by the choice of experimental material. He insisted that his students followed the highest standards of scholarship, making sure that all citations were based on a detailed study of the original material, and he expected an accurate, succinct style of writing like his own. Pat and Peter both remember that this could be very challenging. We may not have reached his high standards, but the trying did no harm. The research output emanating from this school took polychaete endocrinology and the study of reproduction into new territory and clearly demonstrated that there is no such thing as a 'typical polychaete'. The outcome has had a profound influence on the current agenda in polychaete science.

Away from the laboratory, Bob was always generous in his hospitality - Professor D.I.D. Howie, on learning of the passing of Bob Clark, recalled the excellent hospitality he enjoyed at the home of Bob and Mary Clark during the first neurosecretion conference held at Bristol University and commented on how this experience stimulated his own interests in neurosecretion and endocrinology of the lugworm *Arenicola marina,* which, in this way, also entered into the canon of experimental endocrinology of polychaetes. Bob's hospitality was extended not only to his peers, but also to his research students both at Bristol and at Newcastle where, during 'polychaete discussion groups' held at Bob's house in the evenings, with a glass of wine or beer to aid proceedings, his many research students learned to engage in the cut and thrust of scientific debate, to

state clearly, and defend their emerging ideas – a perfect grounding for their later careers.

Bob Clark was a stimulating teacher, expecting students to reach the cutting edge of science and to pursue their own ideas. He held temporary posts at the University of California (Berkeley) and was visiting lecturer at five Western Canadian Universities (Sept. '78 – March '79). Colin Hermans has told us of how Bob's period at Berkeley had a long lasting though largely unseen influence. At Newcastle, as previously at Bristol, he had a strong influence on degree programme development, emphasising the role of University teaching not only in imparting knowledge "but more importantly, to give students the ability to think independently, to form judgements which they can justify and support with tested evidence" (preface to Clark, 1986). Peter and Pat were both student demonstrators to his practical classes, supporting his course on comparative morphology. The labs required a not insignificant understanding of mathematics, and Pat remembers classes, in which nereidids were encouraged to swim, and the students being asked to get a handle on how the worms moved forward – although of course if they try too hard they go backwards – a challenging practical for students and demonstrators alike. Bob also promoted teaching in other areas in which he had an active research interest. He appointed former students S.M. Evans to teach animal behaviour and D.W. Golding to teach endocrinology and neurosecretion and each of them proceeded to create their own research schools in these fields. He appointed Fu Chiang Chia to teach developmental biology taught later by Peter Olive. These appointments ensured that the curriculum for Zoologists and Marine Biologists at Newcastle continued to reflect his influence. He instigated honours student projects, requiring them to carry out investigations of a real problem under the guidance of a member of staff, and to write up their results in the form of a research paper. Peter, who was at Newcastle University as an undergraduate when Bob first burst onto the scene, remembers having to carry out three projects, one comparing the muscular and segmental anatomy of nereidids and glycerids in relation to their mode of life and learning from him trichrome staining techiniques for the histology required.

Bob entered enthusiastically into the scientific life of the North East England. He was an active member of the Natural History Society of Northumbria, taking great interest in the Hancock Natural History Museum, (now Museum of the North), he served for many years as a committee and council member, advising, for instance, on the management of the Farne Islands, as well as being a successful and influential editor of the *The Transactions of the Natural History Society of Northumbria* (1988-1997). He was honoured by election to a Fellowship of the Royal Society of Edinburgh in 1970. His lay interests in Newcastle included his active participation in local education at all levels, he was a governor of Newcastle Prep School, and a well remembered Church Warden at St Georges Church, Jesmond. The vicar at the time, Canon Michael Middleton, recalled at the service of remembrance held at the Church, that Bob brought the same attributes – foresight, clear decision making and a dry wit - to his work for the Parish, just as in his professional life. Peter and Pat have received many emails from friends and former colleagues who recall with warmth his wit and humour as well as his foresight, and excellence as a scientist, scholar and writer.

At Newcastle his interests expanded to include the important field of Marine Pollution. There were at the time a number of serious oil spillages affecting UK coastal waters – most notably the Torrey Canyon in 1968. His response was incisive and practical; he established with John Croxall, an oiled sea bird research unit, to develop a methodology for removing oil from affected sea birds (Clark, 1984; Clark and Croxall, 1972) and, realising the need for a medium for rapid communication of results, he began the series of, at first mimeographed, newsletters, which eventually developed into the leading journal in the field – *Marine Pollution Bulletin* – that he edited for 25 years (see footnote [a]). He was appointed to the *Royal Commission on Environmental Pollution*, working on the 8th RCEP report *Oil Pollution in the Sea*, the findings of which he published in a hugely influential paper in *Transactions of the Royal Society* (Clark, 1982). He travelled widely, investigating and advising on the effects of oil spills around the world (Clark, 1985, 1987, 1991; Dicks *et al.,* 1982, Larmine *et al.,* 1987, Mann and Clark, 1978; Wu and Clark, 1983; Sell *et al.*, 1995). His unrivalled knowledge of marine pollution, clear objective analysis and his characteristic direct writing is shown in the text book *Marine Pollution* (Clark, 1986), which he saw through five editions (the last in 2001). As a text book it is an exemplar of concise, lucid writing which remains the ideal introduction to the subject. Bob continued his work as a marine environment consultant long into retirement. His development from polychaete biologist to world expert in marine pollution, with special expertise in relation to biological impacts of oil spills, presaged by some 30 years, subsequent developments at Newcastle University, where marine sciences, oceanography and marine zoology together with marine technology, naval architecture and offshore engineering now form a unique School of Marine Sciences and Technology (www.NewcastleMarine).

Bob Clark was one of the outstanding scientists of his generation - a great scholar and writer, fondly remembered not only for his scientific work, but for his dry wit, good humour and friendship. In recent years Peter Olive was able to share time with Bob, who would chat over a coffee and a glass of wine or whisky about the old days. He retained a keen interest in the work of his former students, even when increasing infirmity limited his ability to get out and about. Pat remembers being a surprise guest at his 75th Birthday when she again greatly enjoyed his hospitality. Bob's role as a mentor and supporter helped greatly in her move to the Australian Museum, enabling her to take forward polychaete studies and to become involved in the management of the environments Bob had done much to protect. Peter was even able to update Bob on the hosting of the 11th International Polychaete Conference in Sydney (2013). On behalf of the polychaete community, we extend sympathy to the family and friends who survive him, and we hope that our thoughts and remembrance at least recall for others the significance of his scientific life.

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Elis Wyn Knight-Jones: pioneering marine biologist and polychaete taxonomist (1916–2012)

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Figure 1. Wyn Knight-Jones. A, photograph with his mother Maud. On the reverse side, his father wrote "Taken circa May 1916 at Hanford, Stoke on Trent & sent to me in France!"; B, in school photograph, circa 1922; C, with Grandfather, circa 1929.

Wyn Knight-Jones was born in White House, Hanford, Trentham Rural District (now a southern suburb of Stoke-on-Trent) in Staffordshire on 7 March 1916. He was the first child of Maud Knight (née Cotterill) and William Ellis Jones; younger brother Owen Arthur was born in 1923. His mother was one of the first women to obtain a Bachelor of Arts degree in Classics (First Class) and, as an external student, followed this with a Master of Arts degree from the University of London in 1907. His father attended University College of North Wales (now Bangor University) and began a career in banking as an accountant in the London City and Midland Bank in Bala, 50 miles to the southeast.

However, at the time of Wyn's birth he was serving as a Lieutenant in the 14th Battalion Royal Welch Fusiliers in the snow-covered trenches of northern France. A few months later he received his first photograph ("Taken around May") of Wyn and Maud at Hanford (fig. 1A). Unfortunately, he was wounded in early June. William wrote a letter (postmarked June 9) to his father Owen from No. 8 General Hospital, Rouen, telling him that the operation to remove two pieces of shell from his (lower back) wound had been successful. After making a good recovery, he was promoted to Captain and returned to Britain to train young troops, before returning to serve in the Army of the Rhine after the Armistice of 11 November 1918. He was demobbed after Easter 1919 and returned to Bala to forge a successful career at the Midland Bank.

Wyn had no recollection of seeing his father in uniform. One of his earliest memories, at the age of 3, was of his genial paternal grandfather warming his posterior in front of the fire at the Agent's house *Bryngwyn* on the Peniarth Estate, Llanegryn in Gwynedd. Owen Jones and the family lived there from his appointment as Estate Agent in 1890 until his death in 1922. Wyn recalled that his father's brother, John Owen Jones (1884–1972), "alternated between school teaching (chemistry) and professional singing"; in newspaper clippings, he was referred to as the "popular artiste" Owen Bryngwyn.

School (1922–1933)

Banking took Wyn's father to the Head Office of the Midland Bank in London. In 1922, Wyn was part of the Kindergarten at Oakland House School, Blackheath in the southeast of the city, but after the summer he was in the 'Transition Division' at Clanricarde House School (fig. 1B) in Sutton in the southwest. His first report was good; "A very keen pupil. Shows decided aptitude for Drawing and has done well in every way."

In 1926, aged 10, he started boarding at Fonthill Preparatory School, East Grinstead, Surrey. The school wrote to his father in May re-assuring him that Wyn was "settling in

wonderfully well" and making friends. A letter from Wyn to his mother was also positive, but he complained "we scarcely ever go out" and mentioned thinking of murder regarding the lady who came to wake him in the morning! By December, Wyn had clearly settled in and was doing very well academically, though the Headmaster (Rev. Walpole E. Sealy) wrote that "Wyn's love of jests — apparently mistimed? — lost him his Class Prize this term." The next report in April 1927 noted that Wyn seemed to have taken the previous comments to heart, "sensible boy." There were criticisms again in the August report, but that December he won the Class Prize. This up and down pattern was frequent throughout Wyn's remaining time at Fonthill, although in December 1928 the Headmaster grudgingly acknowledged that appendicitis could have affected his work that term. Nevertheless, he managed to pass the Common Entrance Examination for Epsom College in the summer of 1929. The notification of this in early July happily coincided with his father's promotion to General Manager's Assistant at the Midland Bank. Wyn also showed signs of ability in singing, handicrafts, cricket and hockey during his time at Fonthill. In his last school report (August 1929), Rev. Sealy noted that Wyn had come 4th in the class, "which was none too bad". However, he followed this with a warning: "He is of course more capable than he likes us to imagine, and should do well in life: but he must not postpone his efforts too long, or he will find himself a bearded old man boring his grandchildren with the great things that he might have done, if he had been more ambitious."

Wyn started attending Epsom College following the summer break (fig. 1C) and his reports soon followed their usual pattern. They were at first poor, though his potential was readily recognised. His marks in Latin were a good barometer of his progress and response to criticism. In November 1929 he was 19th in a class of 22, yet only a year later he was 3rd equal. The Housemaster wrote "I rejoice to hear of increased effort at Latin and hope it will spread to other subjects." By March 1931 He was 1st in Latin, but improvement was still desired in other areas – and in later reports the need to make more effort was frequently voiced. Wyn responded and his reports from late 1931, and throughout 1932, were good. Conversely, in December, his teacher's comments regarding Zoology were worrying: "Weak. I can hold out no hope for his obtaining a scholarship unless he makes a stupendous effort and vast progress." Concern at his lack of academic application continued in 1933. Nonetheless, Wyn continued to fulfil his potential in other areas. He gained 1st string athletic colours, played in the 3rd XV rugby team and obtained his Bronze Medallion from the Royal Life Saving Society. In addition, in November 1932, he received his Certificate 'A' for the Infantry syllabus in the Junior Division of the Officer Training Corps. In his last Epsom report, in July (aged 17), his Housemaster wrote "Has taken life too easily lately but has done some useful work as a prefect." The Headmaster was more encouraging and wrote that he was a good and promising boy, who should do well, and he wished Wyn well for the future.

University (1933–1939)

"If you're keen on zoology, you'd better go to Bangor" was his father's advice (Knight-Jones, 1996). Wyn easily passed a 'scholarship interview' with Professor F.W. Rogers Brambell FRS and became a student at the University College of North Wales, regardless of there being no actual 'scholarship' available. Brambell had been a prime mover in establishing a Marine Station at Menai Bridge (Psalti, 2001), and the two quickly developed a lasting friendship. True to form, Wyn admitted that it took him another 3 years "to become even moderately studious." Wyn enjoyed university life to the full and took part in athletics, rugby, and rock-climbing. He was awarded full colours for boxing, representing the University of Wales at the Universities Athletic Union (UAU) finals of 1937. In March 1935, he received his Certificate 'B' qualification in the Artillery syllabus of the Officers Training Corps (Senior Division). Wyn also joined a number of university clubs and societies. He was Secretary of the Chess Club, Student President of the Biological Society, and took part in several productions of the English Dramatic Society. Wyn met fellow student Mary (Luned Mary) Morgan-Jones and they began dating (fig. 2A, B).

The mischievous side of his personality was well expressed and some of his exploits gained him a notoriety that was long remembered in the university. One such anecdote implicated Wyn in the release of Cabbage White butterflies (obtained from the Zoology Department) into the projector beam at a local cinema. Two other tales were recounted in the book published to celebrate 50 years of Marine Science Laboratories at Bangor University (Psalti, 2001: 24): "Knight-Jones broke into Powys Hall just before the exams and replaced the blotting paper with toilet tissue. On another occasion he disturbed a ladies' garden party by staging a fight with a friend on the roof overlooking the College garden, then proceeding to throw him down from a considerable height. Only later was it revealed that this was a borrowed tailor's dummy."

Despite these escapades (and others), Wyn did start to take his studies more seriously. He was enthralled by things that interested him and by the knowledgeable scientists that he met through Brambell, or through invited lectures to the Biological Society. For the latter, he remembered being particularly impressed by Walter Garstang, first director of the Lowestoft Marine Laboratory and father-in-law of Alister Hardy (Knight-Jones, 1996). Wyn further developed his interest in marine biology by volunteering to assist H.A. Cole at the Fisheries Experiment Station in Conwy during the 1937 and 1938 summer vacations. He also participated in a Marine Vacation Course at the Marine Biological Association's laboratory in Plymouth around Easter-time 1938. Wyn obtained a First Class Honours BSc in Zoology that very same year.

In 1938–39, Wyn had carried out some research into the nervous system of *Saccoglossus* (Enteropneusta) under the direction of Professor Brambell. Following on from this, and on Brambell's advice, he made contact with Professor J.Z. Young FRS at Oxford with a view to developing this work for a DPhil. His preferred location appeared to have been Magdalen College, however, Young advised that obtaining a scholarship to Jesus College was a better option at that stage.

Figure 2. Wyn Knight-Jones. A, with Mary Morgan-Jones at University College of North Wales, Bangor, circa 1939; B, with Mary, digging at Abersoch, northwest Wales in 1940; C, on leave in Brussels, March 1945; D, in dry suit, with daughter Carolyn, circa 1958.

Wyn then applied for (June) and successfully obtained (July) a Meyricke scholarship of up to £100 p.a. to Jesus College, Oxford, supported by positive testimonials from Brambell, R.W. Dodgson OBE (Ministry of Agriculture & Fisheries, Conwy; and close relative of 'Lewis Carroll') and University College Principal, D. Emrys Evans. The 'Scheme of Research', appended to his letter of application, began "To complete my studies of the histology of the nervous system of Hemichordata and Urochordata, and if possible to extend them to Polyzoa, Phoronidea, and Brachiopoda, in the hope that such work might throw further light on the phylogenetic relations of these groups to one another and to the Chordates and Echinoderms." He ended with "I plan to spend two years at Oxford, and to enter for the degree of DPhil." He was accepted at Jesus College in October. His first two papers were published on the settling behaviour of oyster larvae (with H.A. Cole) and on a new record of *Phoronis*. With the outbreak of war, he scarcely completed the Michaelmas term. Wyn and Mary married on December 9, and he was commissioned in the Royal Artillery, joining the Officers Training Corps at Colwyn Bay.

War Service (1940–1946)

Relatively little is known of Wyn's war years. He rarely talked of them, as was common for many of those involved. He served much of the war based in the United Kingdom. He was successively Gun Position Officer, Command Post Officer and Regimental Survey Officer in regiments of the 3rd Division and 15th (Scottish) Division. He attended courses in Physical Training and Artillery Survey, and competed in Divisional boxing and cross-country running. In addition, he was secretary to several Officers' Messes and President of a Regimental Institute. In 1944, he was in service in Western Europe, landing around D-Day+10 (i.e., ca. June 16). He was promoted to Captain and Troop Commander that October (fig. 2C). Wyn and Mary's first son, Peter, was born in November.

Wyn was wounded at the Rhine crossing near Wesel on 26 March 1945. He was in the front of an armoured vehicle when an armour-piercing shell struck. By strange coincidence, reminiscent of his father's wounding in 1916, he was hit by two shell fragments. In Wyn's case the wounds were to the chest,

and he soon found it difficult to breathe and impossible to walk or exert himself. He was invalided home; firstly to Botleys Park Hospital, Chertsey, in Surrey, then to Caernarfonshire & Anglesey Hospital in Bangor. While recuperating, he corresponded with several fellow officers, who affectionately addressed him by the nickname 'Jonah'. The war in Europe was ending and they were pre-occupied with logistical and administrative matters. Once censorship was lifted, they revealed they were in a small village 20 miles NE of Hamburg. Following his recovery (but with shell fragments still *in situ)*, Wyn was Troop Commander in a Training Regiment until he was demobbed (18 April 1946). He was mentioned in Dispatches (for distinguished service) and this was published in the London Gazette on the 4th April.

DPhil (1946–1950)

Wyn wished to complete his DPhil at Oxford. His application for a 'Further Education and Training Scheme' grant from the Ministry of Agriculture and Fisheries (MAF) was successful and, on 27th June 1946, he was awarded £320 p.a. plus tuition fees to complete his DPhil at Oxford. The following month he was appointed Senior Scientific Officer at MAF, but granted leave for his studies. In 1946, he attended the Trinity and Michaelmas Terms at Oxford, conducting his research under Dr William Holmes, before returning to fisheries research for the Ministry. From June 1947 to February 1950 he was engaged (with R.E. Savage and H.A. Cole) in establishing a laboratory at Burnham-on-Crouch, Essex. The main aim of the new laboratory was to elucidate the conditions necessary for the revival of the Essex oyster industry. Indeed, his renewed research on oysters with Cole produced the landmark paper in which the phenomenon of gregarious settlement was first described (Cole and Knight-Jones, 1949).

In March 1948, he received permission from Oxford to change the title of his thesis from 'A Study of the Nervous Systems of Hemichordata and Urochordata' to 'On the Nervous System, Behaviour and Development of *Saccoglossus*.' A year later he tentatively enquired about his status and of submitting his thesis. The Steward at Jesus College informed him that his name had been kept on the books and that "By virtue of the fact that we have to claim dues and fees from the Ministry each term, you are not such an obscure member as you may think, except for one short period when we really did lose sight of you." Wyn completed his thesis and obtained his DPhil in 1950.

Bangor (1950–1956)

Wyn had an interview for the post of Director at the forthcoming Marine Station at University College of North Wales in May 1949. He was one of five shortlisted candidates, but was unsuccessful. Fabius Gross, the Austrian scientist famed for his experiments on the effects of chemical fertilisers on the growth of phytoplankton and fish, got the job (Psalti, 2001). However, in December, Wyn was successfully appointed Lecturer in Marine Zoology (at a salary of £900 p.a.) under Gross. He started work on 1 February 1950. Unfortunately, Gross soon became ill with leukaemia and he died in June 1950, aged 44. Wyn became Acting Director, but was again unsuccessful in becoming Director in 1951; Dennis Crisp was appointed. Many believed his earlier undergraduate exploits at the University had acted against Wyn and that his subsequent designation as Deputy Director was very much a consolation prize. However, the University had to also avoid the nepotism trap as Wyn's father was then Treasurer of the University College.

He contributed much to the early success of the new marine station that was established at Westbury Mount at Menai Bridge in 1952. The original Westbury Mount House was demolished in 2012 to be redeveloped as a new Innovation Centre (SEACAMS), bringing researchers and businesses together. Wyn got on well with the new Director and, in fact, they lived in adjacent houses in Bangor and Wyn's younger son Philip (born August 1948) remembers playing with Crisp's son Graham. Wyn and Mary's daughter Carolyn was born in June 1954.

He was one of a select and hardy band of pioneering divers who began using SCUBA to further their scientific research in the late 1940s and 1950s. This increased access to, and use of, SCUBA ultimately led to the founding of the British Sub-Aqua Club in 1953 (Rogerson, 2013). Wyn recalled his own experiences at this time (Knight-Jones, 1998), "We acquired dive masks and snorkels in 1953 and an aqualung in 1954." Syed Zahoorul Qasim was Wyn's first research student, arriving in October 1954. They collaborated, as a sideline to Qasim's PhD work on primary production, on studying the responses of various animals to pressure. One *in situ* experiment in 1955 saw Wyn observing *Eurydice* on the seabed of Menai Straits, while Qasim above kept the dinghy on station. Wyn had just finished when he noticed the seabed "rushing by at astonishing speed". He surfaced and, seeing the concerned look on Qasim's face, hauled himself aboard without delay. They had travelled a considerable distance south, but managed to row north against the current and found "a kindly eddy" that helped them home. Qasim was the first student from Menai Bridge to obtain a PhD in Marine Biology (1956). He was later (1967) awarded a DSc and played a significant part in founding the National Institute of Oceanography in Goa. In the 1980s he became Secretary to the Indian Government in the Department of Ocean Development, and initiated India's explorations in Antarctica. Wyn's diving exploits became renowned and his leaking dry suit, evidently ill fitting his slender frame (fig. 2D), more often than not resulted in him getting wet and numbingly cold.

Wyn published 17 papers on a range of subjects between 1951 and 1955. These included ciliary beating in Metazoa, invertebrate larvae at Naples, animal distributions in the rocky intertidal and sublittoral, responses of plankton to changes in hydrostatic pressure, and two from his own DPhil studies. His laboratory experiments on gregariousness during the settlement of barnacles (Knight-Jones, 1953) became a classic work in experimental biology (Toonen, 2005), and his insights into metachronism and ciliary beat (Knight-Jones, 1954) was clearly the product of a very original mind. He built a mechanical model from Meccano® to demonstrate ciliary movement to students. Unfortunately, this was no longer working when he showed it to the senior author in the 1990s.

Throughout this period, Wyn remained ambitious for career progression and, in 1954, he applied for the Chair of Zoology at

Figure 3. Wyn Knight-Jones. A, examining seaweed for polychaetes with Phyllis in Russia, September 1996; B, with Phyllis, 2005.

Bedford College, University of London. While this was unsuccessful, he had more luck when interviewed by Council members at the University College of Swansea in 1956.

Swansea (1956–1981)

As the first Professor of Zoology at Swansea, Wyn was immediately tasked with organising the new Department. He delivered his inaugural lecture 'Marine Biology in Wales' on the 4 December 1956 (Knight-Jones, 1957), primarily describing his work, and that of his former colleagues and student Dr Qasim, to date. He finished by mentioning his current staff – Dr Ernest Naylor, recently joined marine ecologist and isopod expert, and Mr Macfadyen "of terrestrial habits" – and outlining the great opportunities he saw for Marine Biology at Swansea. These ranged from fouling organisms in Swansea docks, shore ecology and local fisheries to making use of the Dale Fort Field Centre and diving the clear waters around Skokholm Island in southwest Wales.

Wyn was soon busy and, continuing his work at Menai Bridge on pressure responses, similarly installed tall glass tubes in the stairwell of the Natural Science Building. In addition, he carried out research on the settlement of Spirorbinae, underwater surveys, intraspecific competition, and the biology of cirripede larvae. Marine biology was becoming a major subject matter in the University and a paper with his first research student at Swansea (Phillipu Hewa Don Hemasiri De Silva) marked the start of his career as a polychaete taxonomist (De Silva and Knight-Jones, 1962). Spirorbins were to be a profitable research field both for Wyn and many subsequent students.

Diving was an important activity and the clear waters of southwest Wales were readily accessible for fieldwork. Both his sons and colleagues have similar memories of some of his exploits. Typically, they recall being left alone in the small boat while Wyn disappeared below with the instruction "Just follow the bubbles, old boy." This was rather worrying and no easy task, for he hardly seemed to breathe! Even more colourful escapades ensued. In 1965, the family toured France and Spain on holiday. However, as usual, Wyn was actively collecting at every opportunity. In Spain, he attracted the attention of the Spanish naval police when he inadvertently dived near Franco's yacht. A couple of years later, returning from a successful diving expedition he led to Chios in the Aegean, Wyn had to convince Greek Customs officials that that he was not removing marine antiquities from the country; he was taking only the insignificant little tube worms that were attached to fragments of amphorae! Julie Bailey-Brock later published an account of the Chios spirorbins (Bailey, 1969).

Wyn was generous in providing help, advice and ideas to colleagues and students, and would unselfishly edit and enhance their manuscripts and other writings. He would regularly collect material for his undergraduate practical classes and postgraduate students, and always led by example. Wyn held his student audience's attention through his quiet charm, humour, and droll sometimes risqué delivery. As a professor he was never a 'committee man' and often had to be reminded that he should have been in a particular meeting ten minutes ago! He published regularly and produced 24 papers between 1956 and 1968, including important contributions to the study of intraspecific competition between sedentary marine animals (Knight-Jones and Moyse, 1961), and to the systematics of marine leeches (Knight-Jones, 1962).

However, family life changed in October 1968 when he and Mary divorced. A relationship subsequently developed with Phyllis Fisher, a keen SCUBA diver he had met at a field outing at Dale Fort (Mackie et al., 2011) and, after a whirlwind romance, they married in July 1969. Phyllis quickly took an interest in his work. She was made a Research Associate at the University in 1970, and the following year accompanied Wyn on a two-month research and teaching visit to South Africa. Wyn was interviewed by the *Cape Times* and, talking about their work on spirorbins, said "It's ridiculous that these creatures should be our bread and butter, but we are quite hooked on them now." This visit was followed in later years by collecting trips to many countries and together they became the foremost taxonomic experts on this group of polychaetes.

The birth of their daughter Gaynor in July 1972 did little to slow them down. They took a cruise from Lisbon to Funchal,

Las Palmas, Tenerife and Lanzarote in 1974, and another from Casablanca to Gibraltar in 1976, gaining access to collecting opportunities at ports in the Canary Islands, Senegal, Sierra Leone, Cape Verde Islands, Madeira and Spain. In the following years, fieldwork was generally more modest, with Phyllis obtaining her MSc in 1977 and her PhD in 1980 from Swansea, and Wyn being awarded a DSc from Oxford in 1977. Then, they finally realised their long-planned South American trip, collecting in Brazil, Argentina, Patagonia and Peru from February to April 1981 (Knight-Jones and Knight-Jones, 1991).

In May 1980, Wyn had written to the University College Principal asking to retire in 1981, when he would be 65. The reply noted that the normal retirement age was, "as you know, 67" and his request to retire early would have to be approved by College Council. This was granted and Wyn officially retired in October 1981. A meeting was held in his honour at the Linnean Society in London on 16 December 1982. The Proceedings, titled 'Biology of Marine Invertebrates', were published in the *Zoological Journal of the Linnean Society* in 1984, and included 17 papers and an introduction by the editor (Ryland, 1984). The Department of Zoology in Swansea presented Wyn with a bound volume.

Retirement (1981–2002)

On retirement, Wyn showed little sign of taking it easy. He and Phyllis always welcomed fellow scientists and friends to their house *Bryngwyn* on Gower, South Wales. Together, they continued to work and publish on polychaetes, collecting at locations in Britain and abroad (Mackie et al., 2011). They successfully applied for an Anglo-Australian fellowship from the Royal Society and, in 1983, embarked on a three-month collecting tour of Australia, taking in the *First International Polychaete Conference* (IPC) at the Australian Museum, Sydney. Phyllis attended the first five Polychaete conferences (1983–1995), but Wyn did not always accompany her – saying someone "had to stay and look after the cat." However, wherever possible, they would go together. Travels abroad included Turkey (1987), Faroe Islands (BIOFAR Symposium), Sweden and Norway (1991), France, plus Fourth IPC (1992), New Zealand and Hawaii (1993), Iceland (BIOICE project, 1994), and Russia (fig. 3A), also attending the *31st European Marine Biological Symposium* in St Petersburg (1996). Wyn was still certified as fit to dive aged 77, but was restricted to sheltered conditions and a maximum depth of 20 m.

Wyn supported Phyllis (fig. 3B) throughout her art project on the Welsh Slate industry and during her illness at the turn of the century (Mackie et al., 2011). At the same time he himself was finding it harder to concentrate, but he persevered and completed his last scientific paper (Knight-Jones and Knight-Jones, 2002).

Health (2002–2012)

Wyn remained in good health throughout the 1990s. Photographs of him at home and on fieldwork show him as active as ever. One photograph from 1997 shows him sitting on the ridge of the roof at *Bryngwyn*, busy repairing the chimney! His last known fieldtrip was to Salcombe and Looe, southwest England in October 2003, only a few weeks after a hip replacement operation. In May 2004, he was elected a Fellow *Honoris causa* of *The Linnean Society of London*. His last driving licence was issued in December 2003, however, there were increasing signs of ill health and in 2005 he had his first visit to a memory consultant. As his health deteriorated over the following years, he was nursed at home by Phyllis. Plans were made for them to join their daughter Gaynor and family at Sale, near Manchester. Unfortunately, Phyllis' health was failing also and she passed away in January 2009. Wyn, suffering from Alzheimer's Disease, moved to Sale and was lovingly cared for until his death from pneumonia on the 9th February 2012, a month short of his 96th birthday.

Reminiscences

Many letters and e-mails were received following Wyn's death. These cannot all be included here; however, those that are provide an insight into the respect and affection he received from all who came in contact with him.

His influence on his former students was everlasting. Pete Vine wrote "He was a really important figure in my life and I shall never forget the encouragement he gave me." Julie Brock recalled learning "to dive in Abereiddy quarry, collecting slate with spirorbids on them. At times it was very cold and Prof would sit in the boat after a dive, drink his bottle of cold water and munch on an apple. He will always be a very gracious and patient advisor."

Those who visited him at *Bryngwyn* had similar thoughts. Nechama Ben-Eliahu: "Years ago I visited with them both in Swansea, and it was an important and memorable visit for me." Tara Macdonald: "He certainly left an inspiring legacy – and I won't forget his excitement about his work, even well after retirement. He was a kind man, and I appreciated that as a young scientist." Chris Mettam wrote that Wyn had a "long and creative life that anyone could be proud of. It was an honour as well as a delight to know him."

Wyn in the field was equally memorable. Mike Kendall: "The abiding memory I have of him comes from the late 70's when he and a field team, all dressed in wet-suits, called into the Robin Hood's Bay lab for coffee totally unannounced. His arrival totally panicked Jack Lewis who wasn't used to that sort of thing, but amused the rest of us." Greg Rouse: "I'll always remember the time I met him and Phyllis at Heron Island. I was new to polychaetes but they were kind to me. Wyn impressed me immensely in that he carried their SCUBA tanks out the reef crest, more than 500 m! He was in his mid-60s at the time." Helmut Zibrowius remarked on Wyn's energy: "I met them last in the Faroes (BIOFAR Symposium) in 1992. Wyn then was still agile in the tidal zone between boulders looking for spirorbids. They were a kind help when young I touched to spirobids (now I am retired, too)." Victor Gallardo recalled Wyn and Phyllis visiting Chile in 1981 and remembered Wyn's humorous comment on "the stately attitude of a humble street dog."

Wyn's qualities were recognized widely. Brian Morton wrote "Very sad. Another one of the 'Greats' gone. But he had a long, productive, active and eminent life." Pat Hutchings and Steve Hawkins were of a similar mind, "He was a scholar and a gent."

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Telesphore Gottfried Pillai (1930 – 2013) obituary

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Telesphore Gottfried Pillai at Gottfried and Toni's 50th wedding anniversary celebrations in 2009 (photograph by Mr. John Cunningham).

Gottfried was born on the 31st. of December, 1930, in Puttalam, Ceylon. His parents meant to call him Godfrey, but the German priest who baptised him entered the name incorrectly in his register. This caused some problems in later life when people wrote to him in German, as he had never learned the language. Ceylon was still a British colony in 1930, but is now independent and since 1972 has been known as Sri Lanka.

Although he was not a lover of all animals (he remembered being trapped in the lavatory by a snake as a boy) he studied Zoology, and Marine Ecology & Fisheries, at university, gaining BSc degrees from the University of Ceylon (1956) and the University of London (1965). In 1970 he gained a PhD in Zoology from the University of Ceylon, with Sir Maurice Yonge as his External Examiner.

It was when he was at University that Gottfried began to seriously study polychaetes as a hobby in the evenings and weekends, and this continued after he entered the world of paid employment. It was also while he was at University that he met his wife Toni. They had three children: Kristina, Johann and Karen. They still remember trips to rocky beaches and the family trailing behind him with buckets and empty baby food jars to collect specimens.

From 1956 to 1968 Gottfried was Superintendent of the Brackish-water Fisheries Development Division of the Fisheries Department, Ceylon, and then from 1968 until his retirement in 1986 he was employed by the Food and Agriculture Organization (FAO) of the United Nations. For the FAO he worked on inland fisheries development in West Irian; lagoons, lakes and aquaculture in Tunisia; aquaculture development in Tanzania; an integrated fishery, fishery biology and fish culture development project in Nepal, and short-term consultancies in Afghanistan, North Yemen and Egypt. In 1982 he became Chief Technical Adviser of the African Regional Aquaculture Centre in Port Harcourt, Nigeria. This was a multinational UNDP project executed by the FAO. It was affiliated to the University of Port Harcourt, for post-graduate level teaching and training of aquaculturists from African countries, besides establishing a Centre for Aquaculture Development and Research.

After retirement he moved to England, and since 1987 he continued his polychaete research as a hobby, at first as a visitor but then as a Scientific Associate of the Natural History Museum, London.

Gottfried's early published works – on the polychaete faunas of Sri Lanka, the Philippines and Indonesia – are still in use today, and the specimens they are based on are often studied by polychaete taxonomists. He separated the family Spirorbidae from the Serpulidae, and Phyllis Knight-Jones named a subgenus (later raised to genus) *Pillaiospira* after him in 1973. His later works concentrated on these two groups, and display his observations of small details in writing and in his beautiful line drawings. He left several unfinished manuscripts, which colleagues will want to edit or complete for publication.

Gottfried was a good man and a committed Christian, attending church almost daily in his retirement. He loved his fellow man. It was his custom, if anyone spoke to him in an unkindly way, to listen and then say "God bless you" before walking on.

Pat Hutchings remembers him as a true gentleman always offering good quality tea in the laboratory at the Natural History Museum and she encouraged him to complete the paper on the serpulid fauna of the Kimberley region in Western Australia based on material collected by her. She remembers having to get his lovely drawings digitised; he never really entered the modern IT world. Pat says "It was a pleasure knowing him and working with him on this large paper which had a fairly long gestation period and he was always receptive to comments".

In 1987 Harry ten Hove decided to spend 10 days in the collections of the NHM, London. He says "Gottfried and his wife Toni kindly offered me hospitality in their home. I remember that as a happy stay, we had a lot of laughs during dinners and the evenings, sharing experiences (not only tots of whiskey) or simply watching one of the utterly British comic TV series. In the Museum and in his home we discussed serpulids widely, often bent over his numerous keenly observed and detailed line drawings. Back in Amsterdam, I sent him the fairly extensive ZMA collections of the genus *Serpula sensu lato*, and he started to study this difficult taxon. His first results on *Spiraserpula* triggered me to search for more material of this overlooked taxon in my as yet unidentified collections from the Canary and Cape Verde Islands and Indonesia, as well as during dives in the Caribbean. Our initial 4 species exploded to 18, almost all new to science (Pillai & ten Hove 1994). When our views occasionally disagreed, he accepted the criticism of his younger colleague graciously. After a carefully phrased but nevertheless particular radical comment on one of his manuscripts from my side he answered by dedicating his next reprint "to Harry with, as usual, my highest personal regards". I will always remember Gottfried as a nice, patient and gentle man, a real gentleman".

Gottfried died after a brief illness on the 24th. of September, 2013, leaving his wife, three children and a grandson.

I would like to thank Toni Pillai, David George, Harry ten Hove and Pat Hutchings—their comments benefitted this obituary greatly.

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Glasby, Wilson, and Hutchings (2003) and other contributors through CSIRO Publishing, provided the award-winning Polychaete Identification Guide CD-ROM over a decade ago. The Guide remains an excellent, if aged, tool familiar to many student and professional researchers alike. The Australian Museum demonstrates similar interactive skill with their newest contribution - the *Invasive Polychaete Identifier. The Identifier* is a clear effort to promote awareness of invasive spionid, serpulid, and sabellid species of Australia (Kupriyanova, Wong, & Hutchings, 2013). Do these invaders bring homogeneous doom to our coast? Or do they prop up biodiversity and ecosystem function? Before researchers can answer such questions they must first be able to reliably separate exotics which are expanding their range from indigenous taxa. Monitoring programmes, our first-line of defence at harbour and mariculture sites, have difficulty contributing to a cohesive national picture due to the widely varying resources available to them. Records from the grey literature, such as technical consulting reports, also have the potential to provide reliable datasets if only it were possible to provide some assurance of identification uniformity. The Australian Museum's *Invasive Polychaete Identifier* attempts to bring some uniformity and order to the chaos and it does so with style.

The Identifier provides a simple, dynamic user interface with engaging polychaete imagery throughout. It seems to have been effectively designed as a compromise for its apparent audience. It doesn't offend specialists with ponderous click-throughs to get to the species lists, yet it also supports workers with limited polychaete experience. Dynamic features were occasionally corrupted so a few workers accessing the Identifier on small screens may find themselves confused at times by disjointed layouts really intended for 1024 x 768 pixel or larger displays. The page design is a bit wasteful of screen real estate, perhaps in an attempt to be less intimidating, but it behaved well in Firefox, Chrome, and IE browsers.

Like so many polychaete workers I've often shuddered at the poorly-preserved mess some field staff bring back to be identified. Since it seems likely that consulting laboratories form a substantial part of the Identifier's audience, I found myself wishing that the treatement of polychaete fixation, preservation, and examination methods was more comprehensive and prominent in the menu structure instead of buried at the bottom of an introductory page. Access to the bibliography and recommended references is tortured and incomplete, but hopefully that will be fixed in the next revision. Overall, the writing is stilted with ambiguous punctuation and grammar in an obvious attempt to be concise, but the essence of the Identifier is its species list, well-documented character states, fabulous imagery, and clear four colour key providing authoritative status for native, cryptogenic, introduced & established, and potentially invasive species. Although the key is clear, humans tend to associate values with colours such as red, yellow, green, and blue so in addition to helping the colour-blind, a more neutral icon system could be deployed in future revisions.

The Identifier provides three ways of accessing the same information. A full species list gives the user quick access to exceptional and frequently annotated illustrations in multiple resolutions alongside clear character statements. It is clear that the authors have taken pains to provide images of the whole animal, anterior features, tube structure, and standout somatic and chaetal characteristics. I found the comparative comments regarding other species which look similar to be enormously helpful. I was also happily surprised by the provision of contact details for specialists - a true testament to the dedication of the authors to providing a valuable service to *Identifier* subscribers . I was, however, disappointed with some of the unqualified comments on geographic distribution. If the intent of the Identifier is to help track species invasions, it is logically counterproductive to encourage technicians to discriminate similar species by geographic distribution rather than character states. The 'Quickfinder' provides access to the same information using a drop-down menu structure whereas a classic pictorial key can be accesed through the unambiguously named 'Identification tool.'

The most useful tool to polychaete neophytes is the gorgeously illustrated glossary. An enormous amount of work

is apparent in the illustrations with a clear eye toward bright and darkfield stereomicroscope users where it would have been easy, instead, to include SEM images so frequently dismissed by monitoring technicians. The authors' technical skill can be most appreciated in the natural colour images of exquisitely mounted specimens with excellent depth-of-field; even when illustrating those pesky soft radiolar features of sabellids. While the pictures are very informative, some of their value is lost when the Identifier provides them in a single fixed resolution. Posting thumbnails linked to higher-resolution images is such a common and useful feature for workers using different kinds of screens that I was puzzled by its sporadic use in the Identifier.

Fundamentally, the Identifier is a response to the ever-present demand by technicians to have good pictures which they can use to process collections quickly and reliably. The illustrations are of unparalleled diagnostic (and frequently artistic) quality and they are extremely valuable even though the 'picture-book identification method' is addictive and inherently dangerous as it can lead to a multi-choice selection process instead of promoting accurate identification practices. The Identifier mitigates this problem by providing clear access to character states which are reliably linked to the excellent glossary. This design will hopefully encourage responsible technicians to associate structures with descriptions and help them develop useful skills in other groups.

The web was invented by scientists and it is in our nature to let information be as free and as accessible as possible, so I am confident that the authors had to put such a useful tool behind a paywall only under duress and pragmatic funding limitations. The pricing structure reflects a clear effort to trade value for value with the end users.

Students are asked to pay a one-time fee of AU$50, while professionals are charged AU$300, and businesses AU$500. I think many workers will be watching to see if this funding model is successful. The cost to a 'developed country' classroom or business is small and if this level of funding is adequate to provide timely updates as more invasives are identified, then the value for money is excellent and well-worth our support in the hopes that the Identifier can continue to develop. Overall, I think the biggest drawback of the Identifier is its isolationist structure. Although publicly accessible databases like the World Register of Marine Species and Encyclopedia of Life can't link to the Identifier, I don't understand why the Identifier doesn't link its users out to such increasingly useful resources.

The *Invasive Polychaete Identifier* is a useful and beautiful tool I will use professionally to identify taxa and as a training utility. The authors and the Australian Museum deserve applause for providing such useful information in a readily accessible and affordable format which is sure to benefit private and governmental monitoring technicians, students, and academics,

Glasby, C.J., Wilson, R.S., & Hutchings, P.A. 2003. *Polychaetes – An interactive identification guide*. CD-ROM ISBN: 9780643067028